PROTOCOL FOR THE DETECTION AND RELOCATION OF FRESHWATER MUSSEL SPECIES AT RISK IN ONTARIO-GREAT LAKES AREA (OGLA)

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ABSTRACT

This document provides general information on the *Species at Risk Act* (*SARA*), implications of *SARA* and its listed species on proposed habitat alterations, and the associated roles and responsibilities of Fisheries and Oceans Canada, Conservation Authorities, proponents, consultants, and other scientific authorities. The purpose of the document is to outline and standardize methods to be incorporated into field surveys carried out to detect, and when appropriate, to relocate and monitor mussel species at risk in Ontario. Guidance on when a *SARA* permit is needed and the process for obtaining the permit is also provided.

This document is designed for use by persons planning projects in and around water that might affect mussel species at risk in Ontario; for example, bridge construction, pipeline crossings, dredging projects, and culvert installations.

RÉSUMÉ

Le présent document fournit des renseignements généraux sur la *Loi sur les espèces en péril* (LEP), sur les conséquences de l'annexe 1 de la LEP et sa liste des espèces sur les modifications à l'habitat proposées ainsi que sur les rôles et responsabilités connexes de Pêches et Océans Canada, des Offices de protection de la nature, des promoteurs, des consultants et d'autres autorités scientifiques. Le document a pour but de décrire brièvement et de normaliser les méthodes à intégrer dans les études sur le terrain effectuées afin de détecter, de surveiller et de relocaliser les espèces de moules en péril en Ontario. De plus, le document indique quand un permis en vertu de la LEP est requis et précise la marche à suivre pour obtenir ce permis.

Ce document est destiné aux personnes qui planifient des projets dans l'eau et en bordure de l'eau qui sont susceptibles de nuire aux espèces de moules en péril en Ontario; par exemple, la construction de pont, les croisements de pipelines, les projets de dragage et les installations de ponceaux.

1.0 INTRODUCTION

1.1 PURPOSE OF THIS DOCUMENT

This document provides protocols and methods for addressing the presence and relocation of mussel species at risk within a survey area. By following these protocols and methods, the project proponent and Fisheries and Oceans Canada (DFO) can determine, with confidence, if a mussel species at risk (SAR) is within the zone of impact, and if the project must be reviewed under the federal *Species At Risk Act* (*SARA*).

Many of these protocols and methods will be necessary components of field surveys conducted to detect, relocate, and monitor SAR mussels in Ontario.

Guidance on when to obtain a *SARA* permit for the collection and handling of species at risk is also provided.

This report includes standardized methods for:

- Conducting a mussel field survey to detect presence or estimate density of mussel SAR;
- Relocating mussels to avoid impacts as a result of a project activity;
- Monitoring mussel survival and growth after their relocation;
- Determining the need for a SAR permit and the process for obtaining the permit.

Figure 1 provides a flowchart to determine the appropriate course of action to address project-specific mussel species at risk issues. The dashed boxes of the flowchart represent the actions that are described in this report.

1.2 WHO SHOULD USE THIS DOCUMENT?

This document is written for persons planning projects in and around water that might affect mussel SAR in Ontario. These activities could include bridge construction, pipeline crossings, dredging projects, and culvert installations.

The protocol provides guidance on methods to detect and relocate mussels in Ontario. The sampling design is one of the most important considerations in this document. The choice of sampling design will be reviewed and approved by DFO. It is important to ensure that appropriate sampling methods are considered in the design of any field survey to detect or relocate mussel SAR.

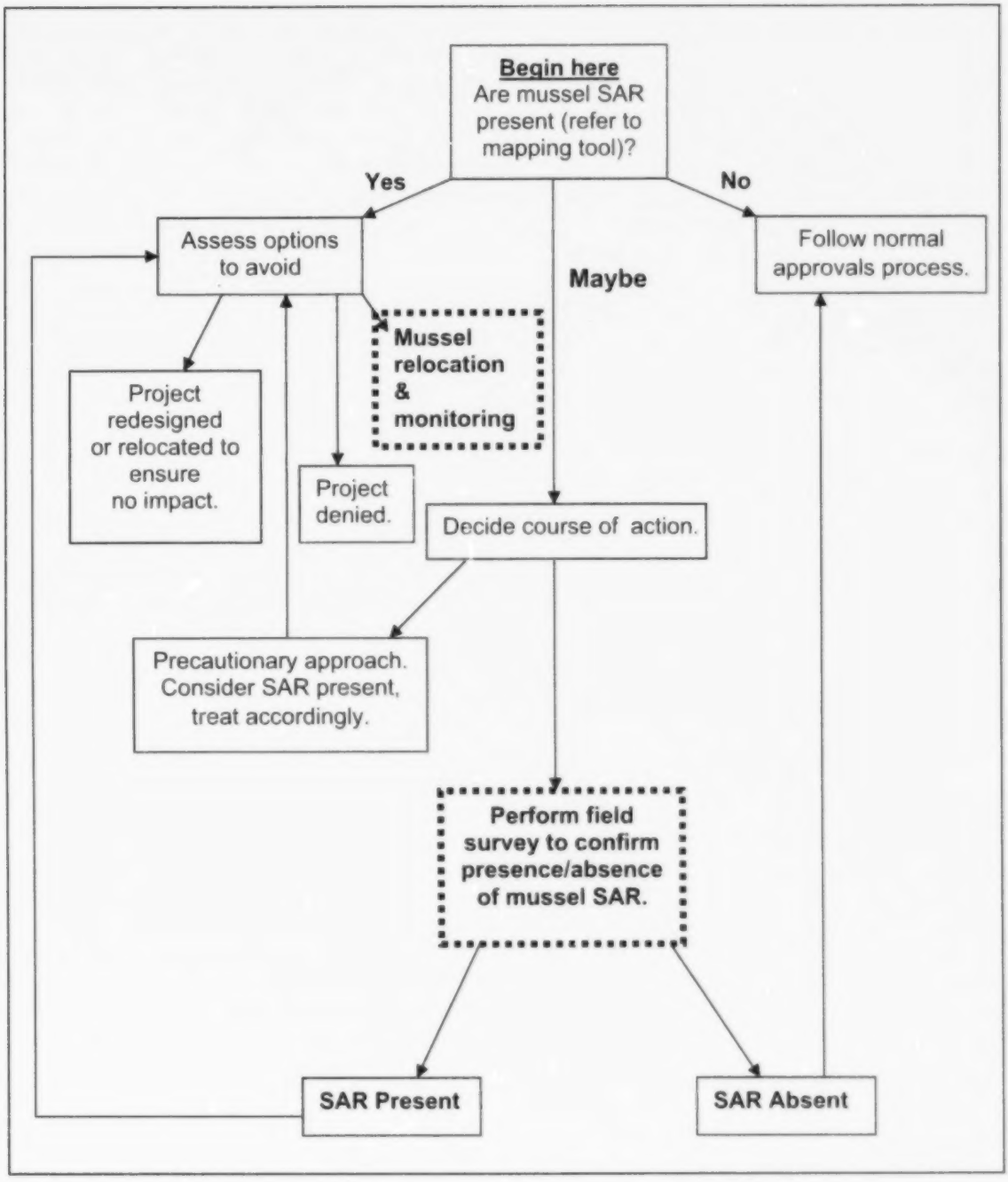


Figure 1. Decision Chart to Determine the Appropriate Course of Action to Address Project-specific Mussel Species at Risk Issues.
(dashed boxes are activities that are described in this report).

1.3 HOW TO USE THIS DOCUMENT

Section 2.0 provides guidance for determining if a mussel sampling program is required. Section 3.0 outlines how to obtain a *SARA* permit. Section 4.0 describes sampling methods for detecting the presence of mussel species at risk. Section 5.0 provides information on recommended techniques for relocating mussels. Details on establishing a monitoring program to follow survival and growth of relocated mussels are also provided. Section 6.0 discusses the necessity of proper documentation of the presence of species at risk and gives guidance on the type of evidence required (e.g., voucher specimen, photographs) for species at risk that occur in Ontario.

1.4 LEGISLATIVE ENVIRONMENT

The purposes of *SARA* are to prevent wildlife species from being extirpated or becoming extinct, to provide for the recovery of wildlife species that are extirpated, endangered or threatened as a result of human activity, and to manage species of special concern to prevent them from becoming endangered or threatened. Currently, several mussels are among the identified species at risk on Schedule 1 of *SARA*. The *SARA* Schedule 1 is the official list of wildlife species at risk in Canada and species on this list are afforded protection by legislation. It includes species that are extirpated, endangered, threatened, and of special concern. Once a species is listed on Schedule 1, protection and recovery measures are developed and implemented. Species listed on Schedule 1 will be periodically reviewed as ongoing studies and investigations provide evidence to justify their inclusion or removal. To obtain a current list of mussel species listed on Schedule 1 of *SARA*, refer to the *SARA* registry website (http://www.sararegistry.gc.ca).

The Minister of Fisheries and Oceans is responsible for aquatic species including freshwater fishes and mussels. Once a species is listed as extirpated, endangered, or threatened on Schedule 1 under *SARA*, it becomes illegal to kill, harass, capture, or harm it in any way. Critical habitats are also protected from destruction. The *Act* also requires that recovery strategies, action plans, and management plans be developed for all listed species. DFO is responsible for the development and coordination of recovery strategies and action plans for endangered or threatened aquatic species at risk.

SARA also amends the definition of 'environmental effect' in the *Canadian Environmental Assessment Act* (*CEAA*) to include any change that a project may cause to a listed species, its critical habitat or the residences of individuals of that species, as defined in *SARA*. Therefore, projects that require an environmental assessment under *CEAA* will have to take into account the project's effects on listed wildlife species and their critical habitats. The assessment must include recommendations for measures to avoid or reduce adverse effects, and plans to monitor the impact of the project if it proceeds. The project plan must respect recovery strategies and action plans.

To determine whether or not the habitat provisions and prohibitions of *SARA* apply to a particular project, it is necessary to know if a species at risk or its habitat will be affected

by the project. For some projects, it will already be known if a species at risk or its habitat is present. For others, it will be necessary to determine if this is the case. Figure 1 is provided to aid in determining the appropriate course of action. Boxes in dashed lines show specific activities for mussel SAR that are covered in this report.

2.0 IS A SAMPLING PROGRAM NECESSARY?

The local Conservation Authority (CA) or DFO office should be contacted to determine if a species at risk may occur in the vicinity of the survey area. An aquatic Species at Risk mapping tool, developed by DFO, compiles all available information on the distribution of aquatic species at risk, and has been distributed to DFO offices and DFO's partner agencies including the CAs, Ontario Ministry of Natural Resources, Ontario Ministry of Transportation, and Parks Canada Agency. These maps are also available on Conservation Ontario's website. Proponents should be working with DFO and/or the appropriate CA to determine the most current distribution of mussel species on Schedule 1 of *SARA*. The mapping tool uses colour segments to distinguish where mussel SAR are likely to be found: red shows SAR on Schedule 1 (threatened, endangered, extirpated); orange shows threatened, endangered, extirpated species that may be listed in one year; purple represents special concern species on Schedules 1 and 3 (for a glossary of terms related to the *Species at Risk Act* go to http:www.sararegistry.gc.ca/). Figure 2 gives an example of a map of known distribution of a mussel SAR in the Grand River watershed. Since all mussels on Schedule 1 in Ontario are either currently listed as endangered, or are under consideration for listing as endangered and threatened, only red and orange zones are shown. The mapping tool is a living document and therefore users should always check for updates prior to beginning a project.

Upon determining that a species at risk may occur at the project site, the next task is to determine, in conjunction with DFO, if a sampling program is required. Generally, sampling to detect the presence of a species at risk will not be required for the following reasons:

- the distribution of the species is known to include/exclude the project site;
- it can be determined, without conducting sampling (e.g., appropriate habitat, mapping tool indicates presence), that there is a very high probability of the species occurring within the project site;
- it can be determined, without conducting sampling (e.g., poor habitat, mapping tool indicates absence), that there is a very low probability of the species occurring within the project site; or,
- it can be determined, without conducting sampling, that there is a very low probability of the species being negatively impacted by the proposed activity.

If a species at risk is known to occur within the survey area, then sampling to demonstrate its presence is redundant and will not be permitted. Similarly, if there is

the potential for a species at risk to occur in a survey area, and the proponent is prepared to assume that the species is present and to allow agencies to assume its presence during project evaluation, then sampling to demonstrate presence is not required. There may be reasons to conduct sampling; for example, to determine the relative significance of specific areas or as part of a monitoring program, but there is no need to sample in order to simply demonstrate presence again, as this would cause additional harm to the species at risk.

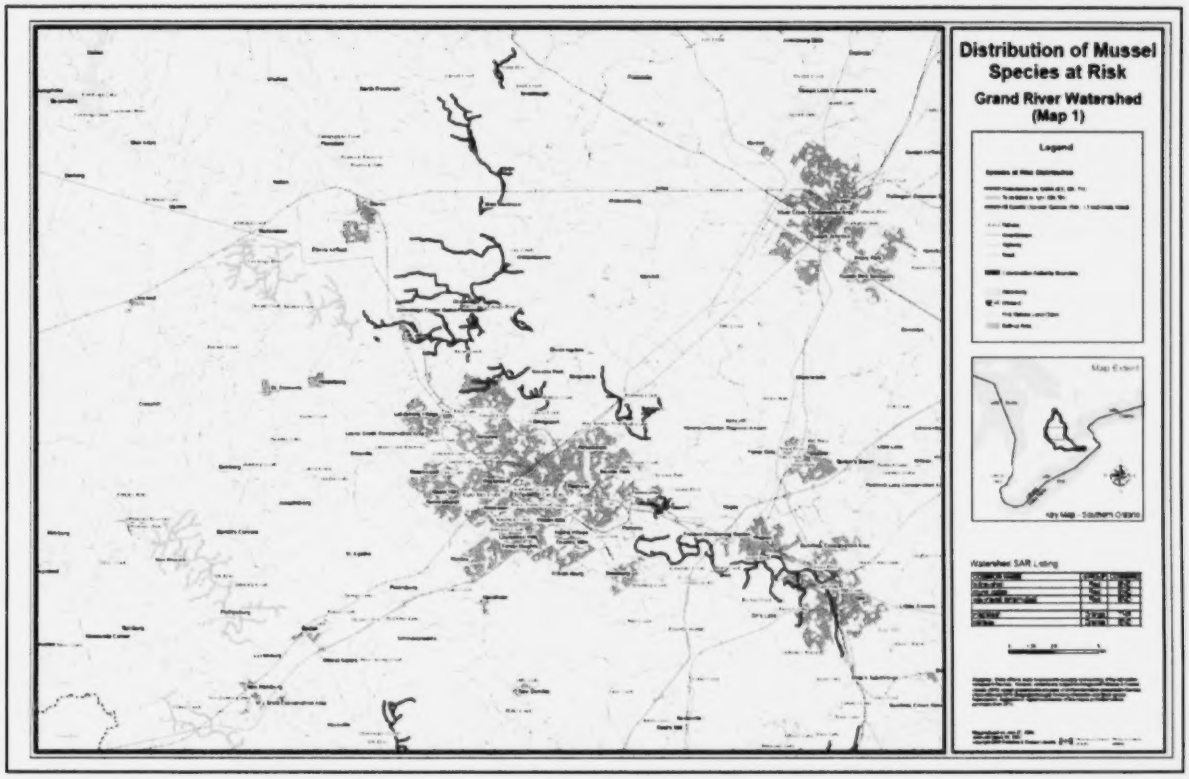


Figure 2. An Example of a Distribution Map of a Mussel Species at Risk using DFO's Mapping Tool for the Grand River Watershed.

There are situations in which the probability of a species at risk being present can be determined by DFO to be sufficiently low without undertaking any field sampling. This may be the case in some areas of southern Ontario, where there is a considerable amount of historical sampling data, and the species at risk has not been previously collected within the study area of the project or habitat conditions are such that a SAR cannot survive.

3.0 OBTAINING A PERMIT UNDER *SARA*

Since *SARA* was enacted on June 1, 2004, it has been an offence under Canadian law to "kill, harm, harass, capture, or take an individual of a listed species that is classed as extirpated, endangered, or threatened". However, activities prohibited under *SARA* may be necessary in order to protect species at risk and there is a provision in *SARA* that allows such activities. At the discretion of DFO they may be allowed if:

a) the activity is scientific research relating to the conservation of the species and conducted by qualified persons;

b) the activity benefits the species or is required to enhance its chance of survival in the wild; or,

c) affecting the species is incidental to the carrying out of the activity.

A *SARA* permit must be obtained if a proposed activity may contravene any one of the three *SARA* prohibitions. These are:

Section 32. (1) No person shall kill, harm, harass, capture or take an individual of a wildlife species that is listed as an extirpated species, an endangered species or a threatened species.

(2) No person shall possess, collect, buy, sell or trade an individual of a wildlife species that is listed as an extirpated species, an endangered species or a threatened species, or any part or derivative of such an individual.

Section 33. No person shall damage or destroy the residence of one or more individuals of a wildlife species that is listed as an endangered species or a threatened species, or that is listed as an extirpated species if a recovery strategy has recommended the reintroduction of the species into the wild in Canada.

Section 58. (1) Subject to this section, no person shall destroy any part of the critical habitat of any listed endangered species or of any listed threatened species — or of any listed extirpated species if a recovery strategy has recommended the reintroduction of the species into the wild in Canada — if:

(a) the critical habitat is on federal land, in the exclusive economic zone of Canada or on the continental shelf of Canada;

(b) the listed species is an aquatic species; or

(c) the listed species is a species of migratory birds protected by the *Migratory Birds Convention Act*, 1994.

This includes any field sampling of species at risk, or fish or mussel salvage operations during a project activity, and is in addition to a provincial scientific collection licence.

The local office of DFO must be contacted (by CAs or proponents) if it is suspected that a permit will be required and DFO will advise.

A permit is obtained by submission of a standard application form. The most recent version of the application form can be obtained from the *SARA* Registry website (http://www.dfo-mpo.gc.ca/species-especes/permits/sarapermits_e.asp). The name and address to whom the application is to be submitted is also given on the website. If it is known that the proposed activity does not pose a threat to the species at risk, or a species at risk does not occur within the area, DFO may advise that a permit is not required.

The applicant must demonstrate that they have sufficient expertise to conduct the field survey, as well as identify the species at risk. A mussel surveyor should have sufficient field experience, including documented field time, and the ability to demonstrate skills in independently executing survey methods, in locating and identifying freshwater mussel species, and in safe care and handling of mussel SAR. Individuals without the experience or education should work with a mussel expert who has experience with the species and field survey methods. Documentation of field-time and/or a letter of recommendation regarding the surveyor's in-basin experience and their knowledge in surveying, handling, and identifying freshwater mussels (including SAR) may be requested. Appendix 1 includes guidelines for filling out an application for a *SARA* Permit as well as an example of a completed application.

4.0 SAMPLING STRATEGIES TO DETECT THE PRESENCE OF SPECIES AT RISK (SAR)

While the presence of a species can be proven, absence rarely can. The most that can be achieved when sampling is to demonstrate that the presence of a species in a particular area is improbable, given the failure to capture by a sampling program in which the probability of capture had it been present, was high. Surveys to detect presence of mussel SAR must be done well in advance of construction activities and proponents must give consideration to a possibility of relocation and the timing of the relocation, ***if required*** (see sections 4.3.1 and 5.2.1). A survey will likely be required if the project is located in a white zone (i.e., no mussel SAR records have been reported) but evidence exists that mussel SAR are likely present. A survey is not required if the project is located in a red zone of DFO's mapping tool distribution maps and DFO may request a mussel relocation. Section 4.0 discusses issues that deal only with a survey to detect presence of mussel SAR; Section 5.0 needs to be consulted ONLY IF a relocation is necessary or possible.

The four key aspects of designing a sampling program are:

- knowledge of the biology of the species at risk (Section 4.1)
- delineation of the survey area (Section 4.2)

- designing the sampling program (locations, gear and effort) (Section 4.3)
- species identification (Section 6.0). Note: This section deals with mussel identification assuming that biologists already have knowledge of the biology, ecology, and physiology of freshwater mussels (see references under Additional Reading, Section 9.0),

4.1 KNOWLEDGE OF THE BIOLOGY OF THE SPECIES AT RISK

The known distribution of the target species, combined with its habitat requirements, must be used to determine if it is reasonable to assume that the target species occurs within the project survey area. Knowledge of the biology of the target species is critical for sampling program design, and for determining if the proposed activity may impact the species at risk. Detailed information regarding the habitat requirements and life history of the target species at risk, including seasonal requirements, is essential in determining the timing, phasing, target habitats, and methods of collection. Committee on the Status of Endangered Wildlife in Canada (COSEWIC) status reports for individual species (http://www.cosewic.gc.ca), as well as recovery strategies posted on the *SARA* registry, are good sources of species-specific information, and often provide a bibliography of additional references. DFO has a series of fact sheets for individual species at risk that can be obtained at local DFO offices or at http://www.dfo-mpo.gc.ca/species-especes/home_e.asp. A search of the recent (post-status report or recovery plan) scientific literature should also be conducted. Relevant habitat and life history information must be included in the documentation of the sampling design.

4.2 DELINEATION OF THE SURVEY AREA

The extent of impacts from the proposed project will delineate the area for which the presence or probable absence of a species at risk must be determined. In projects that only result in direct physical impacts, the survey area may be limited to the project footprint. In projects that also result in indirect impacts such as changes in flow regime, water velocity, water quality, water temperature, siltation, substrate, and bed load, multiple habitat characteristics over large areas may be affected, and the survey area must be sufficiently large to include the area where these potential impacts occur. The survey area must consider both temporary (e.g., during construction) and permanent impacts. For example, the construction of a bridge may potentially result in a temporary, detrimental sediment plume for some distance downstream, as well as a permanent impact to the habitat within the footprint of a mid-channel pier.

The delineated area is referred to as the prescribed search area (PSA) (Figure 3). The PSA consists of: the area to be directly disturbed by a project activity (called an activity zone (AZ)); a risk zone (RZ) that provides some measure of protection beyond the project activity zone and could be directly impacted by accident; and, a zone of influence (ZOI) primarily on the downstream side of the of the AZ that can be indirectly impacted by activities, such as smothering or abrasion from high silt loads.

For example, if the habitat biologist and engineers have estimated that a heavy silt load may impact habitat quality for 7 m downstream, then the ZOI would be estimated at 7 m, as in the example below. The width of the AZ can be determined from engineering diagrams and/or from the proponent's habitat biologist for the project. The width of the RZ can be determined from discussions with engineers and the proponent's habitat biologist on the extent of risks (e.g., heavy equipment turning around) of going beyond the AZ. The width of the ZOI can be determined by the types of protection afforded to downstream disturbance, for example, use of coffer dams, pools, etc. The ZOI may also include an area upstream of the AZ, depending on the activities and types of protection afforded to upstream disturbance. The sizes of the RZ and ZOI are estimated on a case by case basis. Flow also needs to be accounted for since ZOI could increase downstream due to increased flow during spate events. The size of the PSA (= AZ + RZ + ZOI) must be approved by DFO.

For the sake of demonstration, let's suppose the construction zone (AZ) is 18 m wide, the RZ is 5 m on each side of the AZ (=10 m total), a coffer dam limits silt loading and other indirect impacts (ZOI) to an estimated 7 m downstream. The total stream width to be impacted is therefore 35 m; if the mean stream width (length) is 30 m, the total PSA is 1050 m^2.

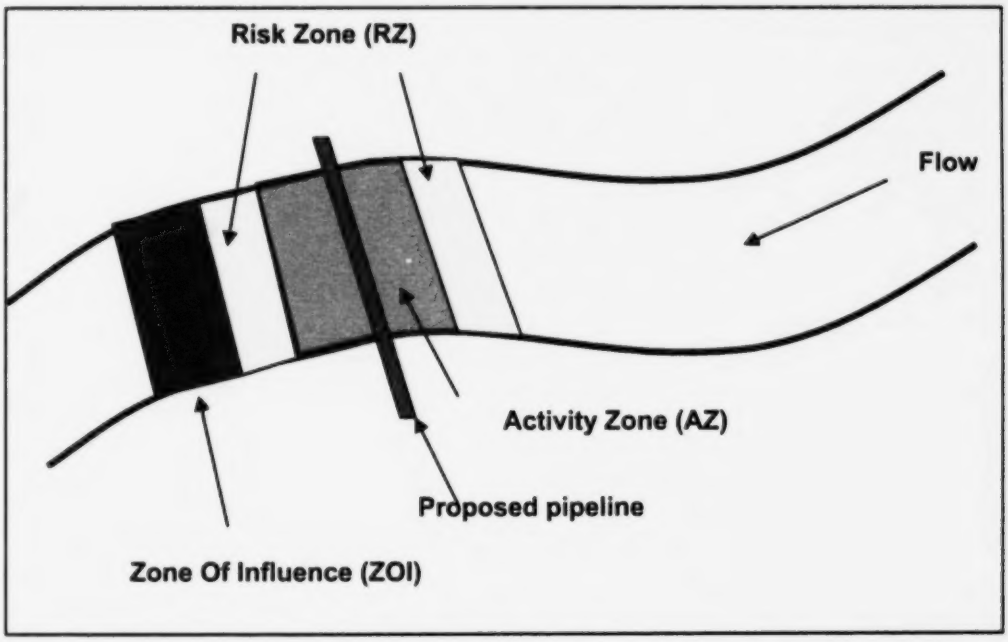


Figure 3. Delineation of the Prescribed Search Area (= Sum of AZ, RZ and ZOI).

4.2.1 Determining Mean and Maximum Depth

The depth measurements enable one to determine the kinds of equipment required to do a survey and if SCUBA divers are needed. SCUBA and/or snorkel divers may be required if depths exceed 1 m. Water depth is also an important criterion in selecting a

relocation site (see Section 5.0), if required. The mean depth can be determined by measuring the depth, in meters, of the stream at 1 to 2 m intervals across the stream on several transects. Minimum depth will always be zero and the maximum the deepest measurement obtained. These depths will change over time depending on time of year, spate events, etc.

4.2.2 Determining Substrate Types and Proportions

Knowing the types of substrates and their proportions will provide knowledge of the extent of habitat types for the SAR in the PSA and are important criteria in the selection of a relocation site (see Section 5.0), if required. At least 10% of the PSA should be sampled randomly using 1 m^2 quadrats, or 105 quadrats (10% 0f 1050 m^2) in our example. The substrate types are usually recorded using the Wentworth (1922) scale : silt to fine gravel (particle sizes <8 mm); fine gravel to coarse gravel (particle sizes 8 – 64 mm); small to medium cobble (particle sizes 64 – 128 mm); large cobble to small boulders (particle sizes 128 – 256 mm); boulders (particle sizes > 256 mm); bedrock (large, extensive areas of solid rock) based on a 15 cm deep sample (Table 1).

Table 1. Sediment Particle Size.

Particle size (mm)	General description
<0.063	silt
0.063-0.125	mud, very fine sand
0.125-0.5	fine to medium fine sand
0.5-8.0	coarse sand to fine gravel
8.0-64.0	small, medium to coarse gravel
64.0-128	small to medium cobble
128-256	large cobble to small boulders
>256	boulders

4.2.3 Water Velocity

Substrate types are usually related to water velocity. Precise measurements of water velocity are not required, but water velocity is an important criterion for selecting a relocation site (see Section 5.0), if it is required. Riffle areas are required by many SAR and the particle sizes often determine the distribution of a species in a riffle zone.

4.2.4 Water Clarity

Water clarity can be assessed visually as clear (substrate visible at all depths), turbid (substrate not visible even in shallow water), or slightly turbid (substrate can be seen in shallow water but not in deeper water). When sampling clear waters, use polarized sunglasses; the polarized lenses help to reduce the reflection of light from the water surface and aid greatly in visually locating mussels. Substrates in turbid waters are most effectively sampled by feel. 'Raccooning' is a term used when sifting gravel and finer substrates between the fingers.

4.3 DESIGNING THE SAMPLING PROGRAM

This section deals only with the protocol to determine if SAR are present in a specified reach of stream (PSA). There are some fundamental concepts that need to be considered, concepts that could affect the desired results, for example, the timing of field studies and the delineation of the survey areas of concern.

4.3.1 Timing of Field Investigations

Spate events, including spring and flash floods, and high water levels will either prevent the beginning a survey or put a halt to in-stream activities. Surveys are ineffective during and directly after spate events and can be dangerous. The best time for collecting mussels is during low flows, when water velocity is at base flow, and turbidity is minimal. Some streams are naturally turbid and tactile collecting methods (e.g. raccooning) must be used, but periods of low flow are still the best times to collect mussels regardless of water clarity. Depending on the spring melt period, the earliest that most streams become accessible in the Ontario-Great Lakes Area (OGLA) are from mid-to-late May or beginning of June.

Surveys should also consider the lower temperature threshold for burrowing activity. Mussels are non-thermoregulators. Mussels should not be disturbed when tissues may freeze during exposure to low temperatures as this may increase the mussel's vulnerability to predation or to being swept downstream due to slower re-anchoring capabilities. There is also evidence (Fuller 1974) that some native mussel species burrow deeply during colder periods, decreasing the likelihood of detection. Sampling can occur between June 1 and September 30 providing water temperatures exceed 16.0°C. Proposed activities outside this timeframe, or water temperature restriction, must be approved by DFO. Proponents need to be aware that the sampling period should allow time for the mussels to rebury themselves.

4.3.2 Survey Approaches

Assuming sampling is required, a choice will have to be made between a qualitative, quantitative, or semi-quantitative survey. Strayer and Smith (2003) provide a detailed discussion of the three methods and should be consulted if the information provided below is insufficient.

Qualitative surveys: Qualitative surveys are designed to detect a mussel's presence without regard to its abundance. The most common qualitative survey methods used are timed searches for live mussels and searches for middens (piles of dead shells left by mussel predators like muskrats and raccoons) on both shorelines. Qualitative surveys are useful for: (1) measuring mussel richness; (2) delineating the range of a species; (3) determining the presence, but not absence, of a mussel SAR in an area of a proposed impact; (4) detecting the presence, but not absence, of rare populations when assessing site-specific impacts on mussel SAR.

Semi-quantitative surveys: Timed-search surveys in known areas are considered semi-quantitative surveys because they combine qualitative (e.g., timed-searches) and

quantitative (e.g., defined spatial extent) methods (see below). Timed-search surveys typically result in more species detections per unit time than do quantitative, quadrat-based surveys (Wilcox et al. 1993; Smith et al. 2001*a*; Strayer et al. 1996).

Quantitative surveys: Quantitative surveys are used to determine the abundance and diversity of mussels in a survey area and typically require excavation and sifting of stream sediments for both adults and juveniles within quadrats. They are used to assess impacts of stressors on mussel populations or to determine changes in population demographics. Quantitative sampling is usually perceived as time-consuming and expensive but yields near perfect search efficiency for rare mussels (McArdle 1990; Green and Young 1993; Hornbach and Deneka 1996; Strayer et al. 1997; Vaughn et al. 1997; Obermeyer 1998; Smith et al. 2001*b*).

4.3.3 Qualitative Surveys to Detect SAR

Qualitative methods using visual and/or tactile timed-searches are more commonly used to detect mussel SAR than are semi-quantitative and quantitative methods. There are two important consequences of a qualitative survey that need to be considered: (1) an activity could be halted if a SAR mussel's presence is confirmed; or, (2) serious negative impacts could result if a survey erroneously concluded no mussel SAR present when in fact there was a failure to detect them. Hence, any qualitative survey should be designed to ensure a high probability of detecting a rare species. The probability of detecting species presence is related to its abundance, its spatial distribution, sampling effort, search efficiency within the area sampled (i.e., detectability), and how sampling effort is distributed within a survey site (Smith et al. 2001a). The fundamental question is, what is the minimal effort required to find a rare species?

The simplest sampling design is a qualitative method known as 'informal' or 'haphazard' sampling (Strayer and Smith 2003). Places for sampling mussels or sediments within a site are chosen without a formal design, for the convenience of the investigator, such as searching for mussels where the habitat looks promising, where visibility is good, where access is convenient, etc. Most timed-searches involve superficial visual or tactile searches to locate mussels at convenient places (e.g., riffles near bridges), or quadrats or transects deployed in convenient or interesting places, or in select locations that look like good mussel habitat. The most serious flaw in informal designs is that it is impossible to make any inferences about an entire mussel population without making the unproven assumption that the samples are representative of the target population (Strayer and Smith 2003). Consequently, results from informal samples are reported without measures of uncertainty. Strayer and Smith (2003) state, "Data collected using informal designs are NOT reliable for assessing population density, relative abundance of species across species or across sites, and temporal changes in mussel populations. Because of these problems, informal sampling is most useful in preliminary surveys and for determining the presence of a mussel species at a site and should be avoided for other applications (e.g., estimating population size, relative abundance, or temporal changes of mussel populations)."

Informal sampling to detect rare species in a given area is recommended by Metcalfe-Smith et al. (2000) and Strayer and Smith (2003) only if the search effort is high and yields high search efficiency. Visual/tactile search times of < 4 person hours typically collected fewer than 50% of the species present (Metcalfe-Smith et al. 2000). Unfortunately, the amount of search effort required to ensure a specific probability of detecting rare species has not been investigated. If the objective does not need to be stated quantitatively, then a survey that provides the total search effort (e.g., total number of person hours) to detect rare species may suffice, but DFO will advise on the search effort required.

For these reasons, **qualitative sampling is not appropriate for investigations relating to development activities**. However, it is discussed here because, when combined with the quantitative approaches outlined below, qualitative sampling may function as an effective preliminary screening tool. A single SAR specimen detected during a preliminary qualitative survey will eliminate the need to undertake the more intensive and expensive quantitative surveys. It will not eliminate the need for relocation if required.

4.3.4 Quantitative and Semi-quantitative Surveys to Detect SAR

When the objective needs to be stated quantitatively, for example, to detect a SAR in a site with a probability of ≥0.95, given that species abundance is ≥ 500, then a formal probability-based protocol, as described below, will be required. A value of 0.95 is selected because it provides for a high probability of finding mussel SAR and it is a standard value used in probability surveys.

One of the more cost effective methods with clear, specific, and quantifiable objectives is the semi-quantitative approach of Smith (2006) that links search area and search efficiency to probability of species detection. Two important elements are required, a minimum threshold for probability (DFO will use 0.95) and an assumed abundance that is deemed appropriate for the survey area (DFO will provide). The form of the relationship is:

$$\text{Probability (detecting at least one individual)} = 1 - eP^{-\beta \cdot \alpha \cdot T/A}$$

where β is the detectability coefficient, or a measure of the search efficiency, which is a function of the species' biology and the selected search methods; α is the estimated area to be searched; T is an abundance estimate; and, A is the PSA. If the density is known, substitute T/A with μ (density of the species). A large number of factors affect the form of the relationship, including biology and natural history of the species (e.g., some are more cryptic by virtue of colour, size, behaviour); vertical position in substrate (e.g., deeper in fall and winter than summer); physical factors (e.g., turbidity, substrate type, vegetative cover); collectors (e.g., experience, fatigue, visual acuity). However, many factors are constant for a species and searchers can cue in specifically on colours and behaviours. Some factors can be somewhat controlled, for example, by selecting experienced people and specific periods of the year for surveys. If the mean density or

percent of samples with a species is known for a SAR in the OGLA, one can provide an estimate of the area needed to be searched for a SAR in a PSA.

For example, assuming 10% of samples in a similar reach of stream have a SAR with an estimated density of $0.1/m^2$; using these data and a PSA of 1200 m^2, then the total abundance is estimated at 120 individuals (e.g., $0.1/m^2$ in 1200 m^2 = 120 individuals). Using a probability of ≥0.95 (tolerance for risk, or allowable harm, as determined by DFO) and a search efficiency, $\beta = 0.1$, as determined by DFO, and transposing above values into the above equation, ($0.95 = 1 - eP^{-0.1 \cdot a \cdot 0.1}$), the area that needs to be searched to find at least one individual is:

$$a = \frac{\ln(1-0.95)}{-0.01} = \frac{-2.996}{-0.01} = 299.6\ m^2$$

or 300, 1 m^2 quadrats need to be distributed throughout the site, preferably within transects oriented perpendicular to shore. Good spatial coverage and balance would be achieved by selecting a random start and placing transects at equal intervals (Smith 2006) from that start. The time required to search the area can then be determined.

4.3.5 Sampling Gear in Shallow Water

The following is a list of gear typically used in shallow water searches for mussels, but if a proponent has gear that is documented as effective or better, it can be used after discussion and approval by DFO. Bold numbers in brackets correspond to images in Figure 4.

- Rubber boots, hip waders, or chest waders, depending on depth
- Rubber gloves (optional)
- $1m^2$ quadrats **(6);** can be smaller if one wants to increase the number of quadrats searched per unit time. Quadrats can be made from copper tubing or other plumbing tubing like ABS or PVC but they tend to be lighter and more susceptible to disruption by currents. The inside measurements must be 1 m x 1 m.
- Glass- or Lucite-bottom viewing boxes **(13).** Can be made from 1.9 mm (1/2") thick plywood with Lucite sealed with silicone in a groove cut into the bottom of all four walls. Before painting to waterproof the wood sides, silicone all joints. The height of the box should be 41 - 46 cm (16" - 18").
- Polarized sunglasses
- 20 L buckets **(8)** for temporarily maintaining mussels removed from the steam bed. Drill numerous 1.5 cm (1/2") diameter holes in the sides for stream water to enter and bathe mussels in the bucket.
- Pond baskets **(9)** (about 15 cm x 15 cm x 10 cm deep or 6" x 6" x 4" deep) with 2 mm square openings for holding juveniles. Place basket inside 20 L bucket.
- Rubber tipped grabbers **(12)** for pulling mussels from sediments in deeper (but < 1 m) waters.

- Sieves (inside dimensions about 36 cm x 36 cm x 11 cm deep or 14" x 14" x 4.5" deep) with 7 mm (diagonal) mesh openings **(10)**
- Scoops **(11)** for shoveling sediments into sieves
- Long handle (1.5 m or 5 feet) mussel scoops with 7 mm mesh **(7)**
- 30 m (100 feet) tape measure **(5)**
- Rebar stakes **(1)** (1-1.5 m or 3-4 feet long, 2 cm or 0.5 inch diameter rebar works well) for marking off search area into 1 m wide by 10 m long rows
- Small sledge **(2)** for pounding stakes into river bed
- Tent peg used for marking locations of mussels **(3)**
- Rope, 10 m lengths to tie to stakes. Tie loops on each end to fit around rebar tightly.
- Rope, 1 m lengths **(4)** to tie to stakes. Tie loops on each end to fit around rebar tightly.
- Etching tools or shellfish tags **(16)** and "Krazy Glue" to code mussels
- Hand-held GPS unit to locate sites (use decimal degrees, report error and datum)
- Calipers **(14)** (2-decimal digital display recommended)
- Field-friendly note book/pad and pencil for recording data (e.g., species, size, etc.)
- Digital camera to photograph unknown species
- Mussel identification manual
- Thermometer **(15)** to measure water temperature. Mussel collections should be made at ≥16.0°C

Any gear preferred by the investigator may be utilized if approved by the permitting agencies and stipulated in the collection licence. However, at the discretion of the responsible DFO personnel, a negative result (no catch of the targeted species at risk) will only be accepted as sufficient to demonstrate its probable absence if the appropriate gear(s) is/are employed, with sufficient effort (see below), under the direction of experienced personnel.

4.3.6 Sampling Gear in Deep Water

The same gear used in sampling shallow water can be used to sample deep water (> 1 m), except scuba gear (e.g., suit, air tanks, regulators, fins, etc.) is included. The same methods and efforts are required as in shallow waters.

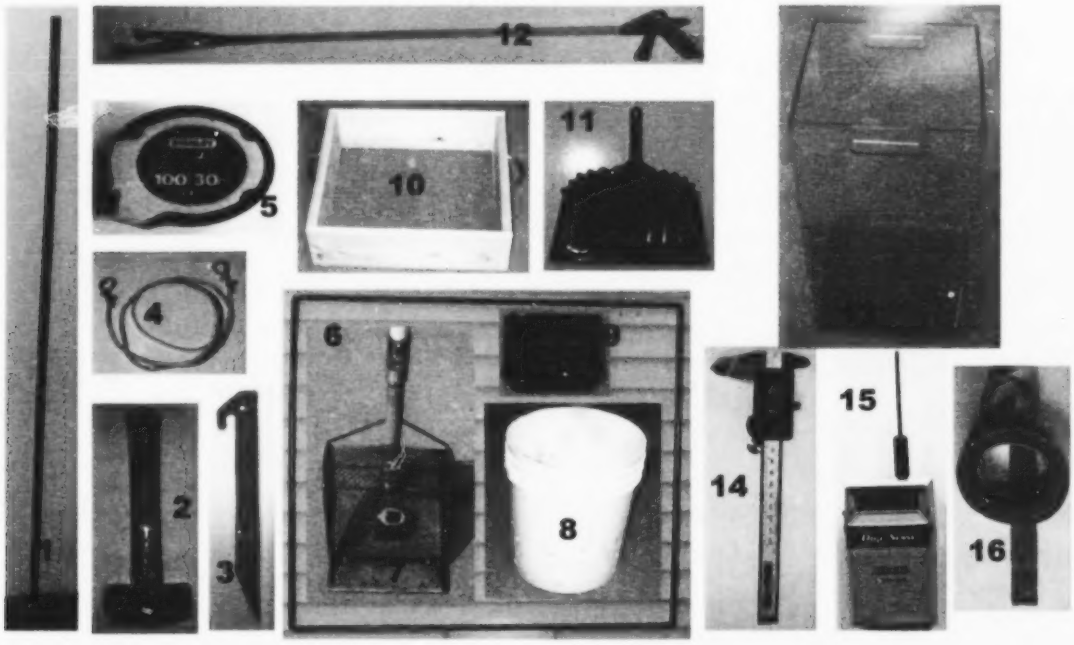


Figure 4. Some Gear Needed for Collecting Mussels in Shallow Water.

5.0 MUSSEL RELOCATION

The goal of a mussel detection program (Section 4.0) is to determine if a species is present or not and, if it is, how widely it is distributed within a defined area. The goal of relocation is to collect and move ALL unionids in a cost-effective manner that will result in high survival of both transplanted individuals and the resident fauna at the recipient site (Havlik 1997). Relocations of mussels must occur within a single drainage area, preferably as close as possible to the construction site to avoid issues with contamination (e.g., parasites, pathogens, aquatic invasive species, genetics). In most cases, the relocation is only a short distance upstream of the construction site. Since a monitoring event (see Section 5.3) must occur one month after the relocation and the water temperature must be > 16.0°C (see Section 4.3.1) during the monitoring event, it is especially important to perform the relocation at least by mid-August, or when water temperatures are well above 16.0°C.

5.1 DELINEATION OF THE RELOCATION SITE

The proponent will be informed by DFO as to whether mussels may be relocated. Usually only one relocation site is required, but if the stream is meandering or braided and a bridge or pipeline crosses it in more than one location, additional relocation sites may be required, including a control site into which no mussels have been relocated.

The control site is needed to determine impacts of relocated mussels on mussels native to the relocation site. The relocation site should be selected on the first site visit. The site visit is necessary to enable an estimate of the time and costs required to reach the work and relocation sites, the accessibility of all sites, and the equipment needed at the sites.

5.1.1 Criteria for Selecting and Delineating a Relocation Site

Relocation sites are usually located upstream of the work site. Transportation from the work site to the relocation site should be kept as short as possible to minimize the time and potential stress on mussels. Whenever possible, keep the relocation site upstream and in the same segment of river as the work site. The relocation site should have both the mussel SAR and its host fishes. Mussel distribution tends to be patchy and mussels should be placed in the more suitable habitat patches of the relocation site. When selecting a relocation site, consideration must be given to the following, keeping in mind that the closer the relocation site is to the construction site, the more similar the habitats, and mussel and fish communities are likely to be:

- Area: The area should be at least the same size, preferably larger, than the PSA.
- Water depth: The depths of water must encompass all the depths from which mussel SAR were removed in the PSA.
- Substrate types: The stream bottom must as heterogeneous and contain all the substrate types in which mussel SAR resided in the PSA.
- Water velocity: The relocation site should have the same extent of riffle areas and pools as the PSA, but substrate types should reflect the range in water velocities.
- Exotic species: There should be no exotic species, particularly dreissenid mussels, upstream of the relocation site.

5.1.2 When More Than One Relocation Site is Required

Most streams meander, but not sufficiently that they are crossed several times by a road or a pipeline. A meandering stream could be crossed by a project at more than one location but the distance between the crossings may be short enough to still justify a single relocation site. In this case the relocation site would be located above the most upstream crossing. If multiple relocation sites are required, ensure that the relocation site(s) for downstream locations are not negatively impacted by the upstream activity sites. The location and number of relocation sites should be approved by DFO.

5.2 RELOCATION OF MUSSELS

The objective is to remove and relocate all juvenile and adult mussels from a potentially impacted area (PSA). Numerous studies have been done on relocated mussels. Dunn and Sietman (1997) suggest the following guidelines to minimize mortality of mussels

while out of the water: (1) use field personnel that are familiar with unionids; (2) select a relocation area with stable substrates and a unionid community similar to that near the collection area; (3) keep animals moist or in water and minimize out-of-water-time; (4) avoid extreme temperatures; (5) avoid overcrowding animals in all stages of relocation (e.g., while maintaining them in buckets and transplanting them into relocations sites). See Havlik (1997) for additional information on the relocation of mussels.

Several studies indicate that the selection of a relocation site should be project specific and should consider the densities of mussels already at the relocation site, the densities of mussels being relocated, and the species assemblages at both the relocation site and the work site.

5.2.1 Timing of Relocations

Section 5.3 outlines procedures for monitoring growth and survival of relocated mussels. Monitoring is required one month, one year, and two years after the relocation. Since mussel burrowing activity declines greatly below 16.0°C, the relocation effort must be done at least one month before the water temperature is expected to drop below 16.0°C. The time of fall when 16.0°C is reached varies from one year to the next but the proponent is expected to allow for a period of mussel burrowing. For Lake Erie, the 16.0°C surface water temperature was reached between mid-September to mid-October between 1994 and 1998. Typically, the relocation should be completed by mid-to-late August, which means monitoring can be done between mid-and-late September.

5.2.2 Collecting Mussels for Relocation

The entire river bottom in the PSA must be surveyed. The PSA should be marked off into rows 1 m wide and perpendicular to one of the shores. If the width of the stream exceeds 10 m, divide the stream into an appropriate number of 10 m or smaller segments. Work side by side beginning at one shore. Continue across the stream until the entire stream width (= PSA length) is covered. Each collector will need (see Section 4.3.5 for details):

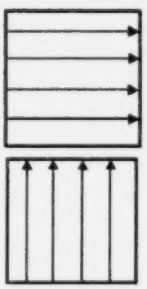
- one 1 m^2 quadrat
- a sieve with 7 mm mesh openings,
- a trowel or scraper (dust pans work well) to scrape top 5-10 cm of sediment into the sieve
- polarized sunglasses if water is clear

Place a 20 L bucket with holes in the stream near shore using rocks to keep it on the bottom and from floating downstream. Use the bucket to hold adult mussels. Place a pond basket inside the bucket to hold juveniles.

Begin by hammering into the stream bed two 1 m long stakes 10 m apart across the stream at the most upstream end of the PSA. Hammer two more stakes into the stream bed 1 m downstream from the first stakes. The use of 1 m and 10 m long ropes with

loops in the ends aids greatly in marking off the transects. Using a 1 m^2 quadrat and starting at the shore in the first row at the most upstream end of the PSA, scrape the top 5 – 10 cm of sediments into a sieve. The sieves have a 7 mm mesh opening and will retain juveniles larger than 7 mm. It may not be possible to identify the juveniles but at least they will be removed from the impacted area. Work toward the downstream end. Some plants and debris will be carried downstream but so will mussels and if the current accidentally carries any out of the sieve, you will have another opportunity to collect them later. Remove large boulders, placing them in an area already searched or area outside of the PSA. Look for large adult mussels first, removing each one and placing it in the holed bucket. Sift the sediments through the sieve. Search the contents of the sieve for any mussels. Remove large rocks and gravel and search for juveniles. Place juveniles in the pond basket inside the 20 L bucket.

Work systematically by beginning in a corner on the upstream end of the quadrat. Work in rows across the quadrat (i.e., perpendicular to shore) with the scraper until the entire same 1 m^2 is searched. Then work in columns (i.e., parallel to shore) along the quadrat until the entire 1 m^2 is searched. Criss-crossing the quadrat will increase the chances of finding mussels that may have fallen off the scraper the first time. When finished, place the quadrat beside the last one in the row. Continue until the entire row has been searched. Using a viewing box, one person should then search the entire row for any adults that may have been missed.



After the first row has been thoroughly searched, move the first pair of stakes downstream of the second pair, leaving the 1 m and 10 m long ropes attached. Repeat the entire process until the second row is searched. Continue until the entire PSA is searched. When a holed bucket is half full of mussels, give it to the coding and measuring crew.

5.2.3 Collecting Mussels at Relocation and Control Sites

The same methods used to collect mussels from the PSA should be used at the relocation site and the control site, except the quadrats can be placed randomly throughout the sites instead of within transects. The main idea is to get some estimate of density and diversity of mussels at both sites. The density and diversity of mussels at the relocation site must be determined **before** any mussels are moved to the relocation site. Collect and sieve the sediments in the same manner as done within the PSA so that both juveniles and adult mussels are collected.

5.2.4 Coding Mussels

Both SAR and a subset of non-SAR mussels need to be coded. The size of the subset will depend on the numbers of non-SAR mussels collected but should be appropriate to statistically determine differences in growth and survival. Code both valves. There are two basic ways to code shells: etching and tagging. Four factors need to be considered in selecting a method for marking shells:

- The well-being and long-term survival of all mussel species being marked – some species have thin shells and are easily damaged by etching methods.
- The period of the survey – the mark should be legible for the entire term of the monitoring studies.
- The position on the shell for ease of observation – the ideal location is near the posterior end on both the left and right valve near the umbo. Selecting a common place makes it easier to find the marks.
- The reposition in the substrate – mussels will bury themselves deeply after a week or two making it difficult to relocate the mussel. Using brightly coloured tags and/or delimiting with coloured pegs such as tent pegs the areas where the mussels were originally placed will facilitate finding the mussels again.

Many malacologists use 8 mm by 4 mm oval Hallprint Shellfish Tags® (Figure 5). Lemarie et al. (2000) found that Hallprint Shellfish Tags® attached with Krazy Glue® (cyanoacrylate) works well. The tags are easy to apply, inexpensive, and provide excellent long term legibility and retention. Waterproof/weatherproof paper or polyester labels (about 6 mm or ¼" diameter) also work well and are available from most stationery stores.

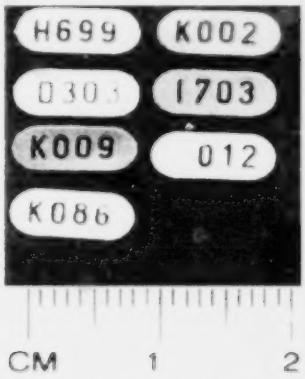


Figure 5. Hallprint Shellfish Tags®.

Cyanoacrylate (e.g., super or crazy glue) adhesives are available from most hardware stores. Although bulk supplies can be obtained from the manufacturers, those made for household purposes have often been found to be superior to special purpose grades. Most important, take extreme care not to get any glue between the gape of the mussel's valves.

To apply the tags, scrape any loose debris from the shell and position the tag on the posterior portion of the umbo, near the dorsal edge of the shell. (Figure 6). Both valves must be tagged. Wipe the shell nearly dry with a dry cloth; effective bonds will form if

- The well-being and long-term survival of all mussel species being marked – some species have thin shells and are easily damaged by etching methods.
- The period of the survey – the mark should be legible for the entire term of the monitoring studies.
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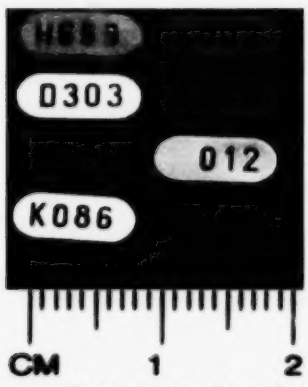

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the shell is just damp but not saturated. Pick a tag from the roll by grasping the edge with the tweezers, smear a little adhesive on the unprinted surface of the tag, place it on the shell and press down firmly, particularly around the edges. The setting mechanism of the adhesive relies on the presence of moisture to neutralize the chemical providing a buffer between the acrylic molecules. Therefore, applying the adhesive to the shell first will see the action almost over before the tag is placed. Use only the minimum amount of adhesive necessary to coat the tag and penetrate the shell. If testing adhesion, leave for a while to ensure that the chemical adhesion activity is complete. The codes used are unique to every person and survey but they should be cross-referenced to field notes that identify species, year, source (e.g., impact, endemic, control), the individual and its measurements. The notes should include sex if individual is sexually dimorphic. If different colours of tags are available, the colour can be used for one datum (e.g., source or species).

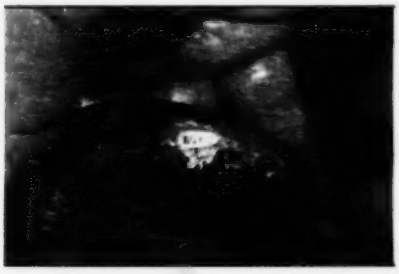

Figure 6. Shellfish Tag on a Kidneyshell.

5.2.5 Measuring Mussels

Three measurements are to be taken for ALL relocated mussels, including non-SAR: length (longest anterior to posterior measurement); height (greatest ventral to dorsal distance); width or thickness (greatest side to side measurement) (Figure 7).

Since monitoring of growth and survival of relocated mussels is to be compared to mussels endemic to the relocation site, shell measurements need to be taken on a representative sample of endemic mussels as well. In addition, shell measurements need to be taken on representative specimens of the same species in areas outside, but near, the relocation site (i.e., control mussels). These measurements are needed to determine impacts of relocated mussels on endemic mussels (i.e., compare growth and survival of relocated species to growth and survival of endemic species and to growth and survival of species in undisturbed habitat).

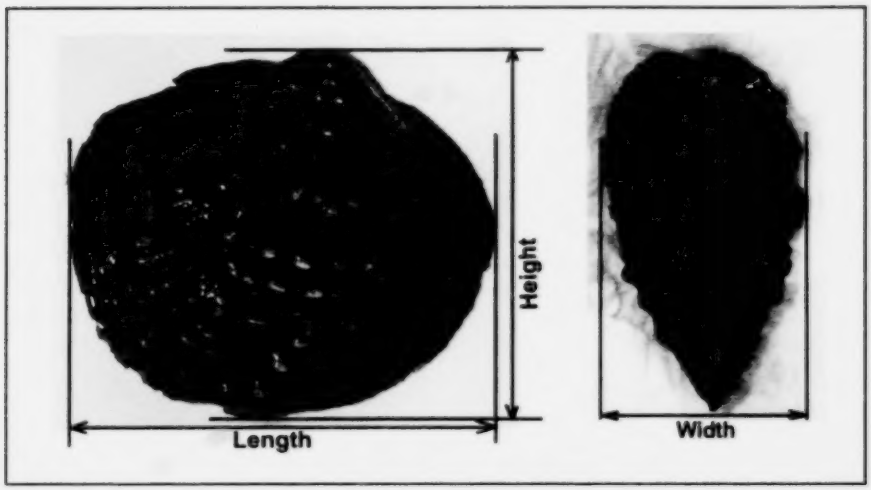

Figure 7. Length, Height, and Width Aspects of a Mussel.
(right valve and end view of *Cyclonais tuberculata* shown).

5.2.6 Replacement of Mussels

In general, place the mussels in the same position as you found them. Using your hands or a trowel, dig a hole deep enough to place the mussel vertically in the hole, posterior (siphons) end up. Replace the sediment alongside the mussel. If the mussel is displaying, lay it on its side; it will rebury itself. Studies have shown that even if the mussel is placed with anterior end up it will right itself, but less stress and energy is spent by the mussel if placed in its normal position (posterior end up). Be gentle with juveniles. The shells are thin and a shallow hole should be dug with your fingers. Place the juvenile in the hole and replace the sediment around it.

5.2.7 Respect for Mussels

All mussels have certain behaviours and physiological tolerances and requirements that need to be respected:

- *Maintenance of mussels while marking and measuring them.* Use 20 L to 23 L buckets with numerous 1 cm (1/2 ") diameter holes drilled into the sides for maintaining mussels in the stream while collecting or measuring them. Place the bucket deep enough in the stream to bathe all mussels and ensure that water is flowing through the bucket. Do not fill more than half full with mussels and keep bucket out of direct sunlight (e.g., cover with a lid).
- *Thermal tolerances and dissolved oxygen requirements of mussels.* Unionids are good water quality indicators and most require saturated levels of dissolved oxygen. The solubility of oxygen decreases with increasing temperature so the amount available to mussels decreases at increasing temperatures. Similar air and water temperatures and maintenance of dissolved oxygen levels between the PSA and the relocation site help to reduce stress during transport and handling. Do not collect mussels when the water temperature is less than 16.0°C

(61.0°F) (see Section 4.3.1). Low temperatures are more of a fall issue because a disturbed mussel will use valuable energy reserves to rebury, if it is are able to at all. In the spring, water soon warms and mussels can then rebury themselves. Upper lethal water temperatures varies among species and exposure periods but > 29°C is considered lethal to most unionids (Fuller 1974) so keep the mussels out of direct sunlight.

- *Effects of handling and air exposure on mussels.* Although exposures to air temperatures between 15 to 35°C for 15 to 60 minutes do not greatly impair mussel survival and over-all stress (Bartsch et al. 2000; Greseth et al. 2003), it is a good idea to limit the period of exposure to high temperatures. The time of year (e.g., June vs. October) also may affect survival and recovery rates, depending upon species.
- *Placing mussels into substrate at the relocation site.* Each species of mussel should be placed in a substrate that is similar to that from which it was removed, following recommendations in Section 5.2.6.
- In summary, proper handling (e.g., limiting air exposure), transport protocols (e.g., keeping water temperature within ±2°C), and selection of suitable relocation habitat with stable substrates are key criteria for ensuring survival of mussels at relocation sites.

5.3 MONITORING SURVIVAL AND GROWTH OF RELOCATED MUSSELS

Monitoring of relocated mussels must occur at 1 month, 1 year, and 2 years post relocation. Monitoring of survival and growth of relocated mussels is required over at least two years to assess the efficacy of relocations. Monitoring must be done when water temperature is > 16.0°C to allow mussels to rebury themselves. Ideally, relocations should be completed by mid-August to ensure temperatures do not drop below 16.0°C one month later or when the first monitoring event will occur.

5.3.1 Frequency of Monitoring Surveys

Short-term (e.g., one month) or immediate impacts of relocations on survival and growth of mussels need to be assessed as well as long-term impacts (e.g., one and two years). If there is a short-term impact, appropriate mitigation actions may be required, such as diluting the density of mussels at the relocation site, or fencing the area off if cattle have suddenly invaded the area, or controlling excessive predation. One would expect mortality of older mussels to exceed that of young mussels and mitigation may not be required if this is the case. If only a few large, old mussels are found dead, there may be no cause for concern. But if many smaller, young mussels have died in the first month after relocation, there is need for concern.

The monitoring events over this two-year period are designed to determine the effects of handling (e.g., coding, measuring, aerial exposure, holding time, etc.) and new habitat structure (e.g., substrate heterogeneity, water velocity, community assemblage, etc.) on relocated mussels and on the native mussel community. This is the

responsibility of the proponent. The proponent must ensure that the short term impacts on relocated and native mussels, if any, are reported after each monitoring event. Appendix 2 gives an example of the kinds of data to be collected and reported.

5.3.2 Increasing the Probabilities of Finding Relocated Mussel SAR

Mussels are much easier to find in summer than in autumn because they tend to be higher in the substrate and their siphons are exposed. Once autumn approaches, mussels begin to bury themselves and recovery rates decrease. High flows also trigger burrowing (New York Power Authority 2005). The chances of recovering mussels can be greatly enhanced by using coloured tags and placing coloured pegs near the mussels.

5.3.3 Measuring Survival of Mussels

Survival is best estimated by looking for marked and coded *dead* shells and subtracting these from the total number of coded *live* shells and expressing as a percent. Alternatively, look for all live shells and if all are found, conclude 100% survival. However, if less than 100% are found, some living shells may have been missed and it is not valid to conclude that the difference represents mortality; it may represent a poor recovery rate. The results must be reported to DFO.

5.3.4 Measuring Growth of Mussels

Unionids grow very slowly and it is difficult to measure significant growth over short periods. Smaller mussels put on more growth than larger mussels in a similar unit of time. However, after one and two years of monitoring, any impacts of relocation of mussels should be discernable. The impacts can be discerned by comparing growth and survival of relocated mussels to growth and survival of native mussels. Impacts on native mussels can be determined by comparing growth and survival of native mussels to those of control mussels.

6.0 IDENTIFICATION OF MUSSELS

In all fisheries work, whether it is a simple mussel community survey, a monitoring program, a population estimate, or a mussel behavioural study, the accurate identification of mussels is one of the most critical aspects of the endeavour. This is equally true in the search for species at risk, as the initial critical step in the protection of a species at risk population and its habitat is the verifiable identification of the species at risk from the mussels captured at a site. The considerable effort often required to establish the status of a species at risk within an area is wasted if the species at risk is misidentified, or even if a species at risk is identified by a competent biologist and the identification cannot be verified if called into question.

The accurate identification of mussels is not easy, and overconfident field workers often miss rare species, or misidentify species because of the over-reliance upon one or two

key identification features. The dichotomous keys that are part of any regional or national text of mussels all require specialized tools (e.g., microscopes) that are unlikely to be available in the field. In addition, many of the diagnostic features are internal and the mussel must be sacrificed to reveal these features. There are also critical points in any identification key that are often misinterpreted during the examination of certain species, leading to a misidentification. Furthermore, almost all keys have been developed using preserved museum specimens, and may rely on pigmentation or other markers that are not readily visible in live mussels.

There are two ways to verifiably identify a mussel species. The traditional way is to preserve a voucher specimen of the mussel. Include a waterproof label in the bottle that has, at a minimum, locality data (location description, and geographical coordinates), date of collection, and the collector's name. The suspected SAR is then submitted to a recognized expert for confirmation of identification. The second way is to provide a photographic voucher of the live mussel, which is then released. The photographs are sent to a recognized expert for confirmation of identification.

For species that are represented by numerous individuals, the specimen preservation method is by far the best, as it provides a specimen that can be examined in any number of ways by experts. This method has a long history of establishing a species presence at a site and is the practical basis of all museum collections upon which species distribution maps, natural history texts, and biological studies are based. A properly labelled and preserved specimen is generally considered absolute proof that a species exists, or existed, at a particular location. Furthermore, preserving a specimen does not require much equipment and is essentially foolproof, since the act of positively identifying the specimen is deferred to when the specimen is delivered to an expert, or any number of experts. The main drawback is that some specimens must be sacrificed. To minimize the number of specimens taken, it is important that someone capable of some level of species identification be involved in the collection effort. This person can sort through the species captured and identify any suspected target SAR, or minimally, be capable of recognizing how many species have been captured, and preserve one adult specimen of each. While one specimen may be inconsequential to a viable population of a short-lived fish SAR, this is not necessarily true for a rare mussel SAR that may have a life span of 30-50 years and sacrificing even one individual may be of significant consequence to the total mussel population.

Photographic vouchers are equally preferred for identifying rare mussel SAR. Photography requires more time, and equipment, as well as a certain amount of mussel handling expertise and photographic ability. A good digital camera capable of macro-photography is recommended in the field. The photographic views required may be different for each mussel as the key identification characters differ from species to species. The photographer must know what the key identification characters are for the SAR so that these can be photographed, and the photographs must be of sufficient quality to allow someone else to positively identify the mussel. Generally, it is easier to photograph a fully grown mussel, but if only small length classes are available, the largest will do. Photography to identify a SAR has several drawbacks; it takes a greater amount of identification expertise during the field collection of mussels and time and expertise are required to properly orient and photograph the mussel. If the photographs

prove to be inadequate, the SAR will remain unidentified as there is no specimen to examine in greater detail. Keep the mussel submersed in water until a photograph is taken so the mussel has a better chance of surviving the photographic process. Minimize handling and the time required to obtain photographs or the mussel may die after it is released. Sensitivity to handling varies from species to species. The only advantage with photography is the knowledge that an individual of a SAR was not sacrificed, providing it did not die during or after the process.

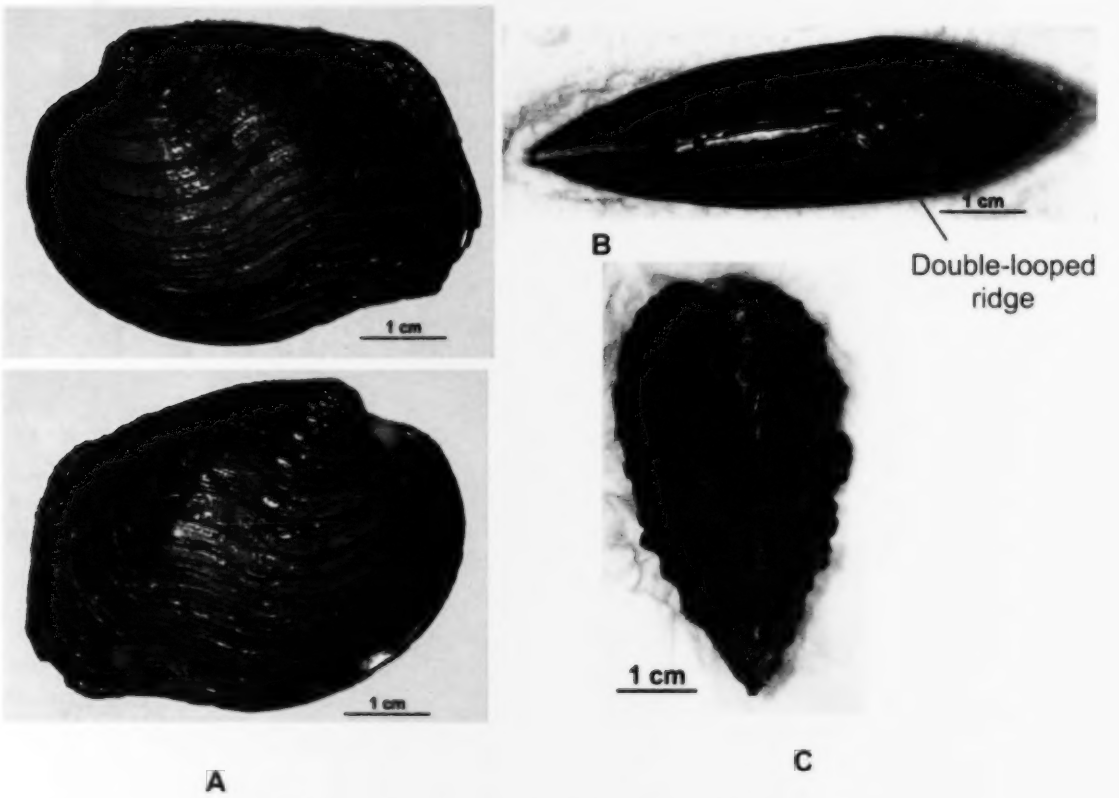

Figure 8. Photographic Views of a Mussel for Identification Purposes.
A. *Quadrula quadrula*, left valve, outside lateral view on top; right valve, outside lateral view below;
B. *Lasmigona costata*, dorsal view showing double-looped ridges;
C. *Lasmigona costata*, end view.

Three colour photographic views of a mussel are usually sufficient to reliably identify it to species: (1) a lateral view (Figure 8A); (2) a dorsal view (Figure 8B); and (3) an end view (Figure 8C). The lateral view must provide sufficient detail to show disc features, for example colour and kinds of rays, pustules, ridges, growth lines, etc. The dorsal view must show details of beak sculpture. The end view must show the degree of inflatedness of the shell, any prominent ridges, and beak prominence. If a freshly dead specimen is available, bring it back so the expert can examine interiors of the left and

right valves, the shape and extent of lateral and pseudocardinal teeth, hinge features (e.g., ligament length), muscle scars, and colour of the nacre. Include a penny or any coin to show size, or measure and include scale bars.

If a specimen is kept for verification of species identity, it is important to preserve it in a way that is appropriate for the suspected species and life stage. All adult and small juvenile mussels should be preserved in 95% ethanol. If specimens are to be used for genetic studies, keep on ice until they can be stored frozen. The anterior and posterior adductor muscles of adult mussels should be cut so that the valves gape and ethanol can preserve the huge tissue mass. A syringe should be used to inject preservative into the body cavity and tissue of small mussels

7.0 SUMMARY AND CONTACT INFORMATION

This document provides general information on the *Species at Risk Act* (*SARA*), legislation, implications of Schedule 1 and its listed species on proposed habitat alterations, and the associated roles and responsibilities of Fisheries and Oceans Canada (DFO), Conservation Authorities (CAs), proponents, consultants and other scientific authorities. The purpose of this document is to outline and standardize methods that should be followed to conduct field surveys to detect presence/density of mussel SAR, to relocate and to monitor mussel survival and growth after their relocation, and to provide guidance on when a *SARA* permit is needed and the process for obtaining the permit.

Information is also provided on when sampling is not necessary and other considerations, for example, mapping tools available for known distributions of Schedule 1 species, importance of site visits, and how to estimate costs for detecting, relocating, and monitoring mussel survival and growth.

The following websites provide detailed information on the *SARA*, COSEWIC, DFO. Websites that describe recovery plans for mussel SAR in Ontario are also listed. The latter also provides contact names of CAs within each venue.

SARA Public Registry: http://www.sararegistry.gc.ca/the_act/default_e.cfm

COSEWIC: http://www.cosewic.gc.ca/

COSEWIC Status Reports: http://www.cosewic.gc.ca/eng/sct2/index_e.cfm

DFO's role in *SARA*:
http://www.dfo-mpo.gc.ca/species-especes/actMeans/actMeans_faqs_e.asp#faq_4

Ausable River Recovery Plan:
http://www.abca.on.ca/documents/Ausable-RS-draft-publicmeeting.pdf

Sydenham River Recovery Plan:
http://www.sydenhamriver.on.ca/

Thames River Recovery Plan:
http://www.thamesriver.on.ca/Species_at_Risk/species_at_risk.htm

8.0 REFERENCES

Bartsch, M.R., Waller, D.L., Cope, W.G., and Gutreuter, S. 2000. Emersion and thermal tolerances of three species of unionid mussels: survival and behavioural effects. Journal of Shellfish Research 19: 233-240.

Dunn, H.L. 1993. Survival of unionids four years after relocation. *In* Conservation and management of freshwater mussels II. initiatives for the future. *Edited by* K S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp.93-99.

Dunn, H. L., and Sietman, B.E. 1997. Guidelines used in four geographically diverse unionid relocations. *In*. Conservation and management of freshwater mussels II: initiatives for the future. Proceedings of a Upper Mississippi River Conservation Committee symposium, *Edited by* Cummings, K. S., A. C. Buchanan, C. A. Mayer, and T. J. Naimo. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp 176-183.

Fuller, S.L.H. 1974. Clams and Mussels (Mollusca: Bivalvia). *In* Pollution Ecology of Freshwater Invertebrates. *Edited by* C.W. Hart, Jr., and S.L.H. Fuller. Academic Press, New York, New York, U.S.A. pp.215-273.

Green, R.H., and Young, R.C. 1993. Sampling to detect rare species. Ecol. Appl. 3:351-356.

Greseth, S.L., Cope, W.G., Rada, R.G., Waller, D.L., and Bartsch, M.R. 2003. Biochemical composition of three unionid mussels after emersion. J. Moll. Stud. 69: 101-106.

Havlik, M.E. 1997. Are unionid mollusk translocations a viable mitigation technique? The Wolf River, Wisconsin, experience, 1992-1995. *In* Conservation and management of freshwater mussels II. Initiatives for the future. *Edited by* K S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp. 184-195.

Hornbach, D.J., and Deneka, T. 1996. A comparison of a qualitative and a quantitative collection method for examining freshwater mussel assemblages. Journal of the North American Benthological Society 15:587-596.

Lemarie, D.P., Smith, D.R., Villella, R.F., and Waller, D.A. 2000. Evaluation of tag types and adhesives for marking freshwater mussels (Mollusca: Unionidae). Journal of Shellfish Research 19(1):247-250.

McArdle, B.H. 1990. When are rare species not there? Oikos 57:276-277.

Metcalfe-Smith, J.L., Di Maio, J., Mackie, G.L., and Staton, S.K. 2000. Effects of sampling effort on the efficiency of the timed search method for sampling freshwater mussels. Journal of the North American Benthological Society. 19(4): 725-732.

New York Power Authority. 2005. Assessment of the potential effects of water level and flow fluctuations and land management practices on rare, threatened, and endangered species and significant occurrences of natural communities at the Niagara Power Project. New York Power Authority, Niagara Power Project FERC No. 2216.

Obermeyer, B.K. 1998. A comparison of quadrats versus timed snorkel searches for assessing freshwater mussels. American Midland Naturalist 139:331-339.

Smith, D.R. 2006. Survey design for detecting rare freshwater mussels. Journal of North American Benthological Society 25(3). In press.

Smith, D.R., Villella, R.F., and Lemarie, D.P. 2001*a*. Survey protocol for assessment of endangered freshwater mussels in the Allegheny River, Pennsylvania. Journal of the North American Benthological Society 20:118-132.

Smith, D.R., Villella, R.F., Lemarie, D.P., and von Oettingen, S. 2001*b*. How much excavation is needed to monitor freshwater mussels? Freshwater Mollusk Symposium Proceedings. Ohio Biological Survey, Columbus, Ohio. pp. 203-218.

Strayer, D.L., and Smith, D.R. 2003. A guide to sampling freshwater mussel populations. American Fisheries Society, Monograph 8, Bethesda, Maryland. 103 p.

Strayer, D.L., Sprague, S., and Claypool, S. 1996. A range-wide assessment of populations of *Alasmidonta heterodon*, an endangered freshwater mussel (Bivalvia: Unionidae). Journal of the North American Benthological Society 15:308-317.

Strayer, D.L., Claypool, S., and Sprague, S. 1997. Assessing unionid populations with quadrats and timed searches. *In* Conservation and management of freshwater mussels II. Initiatives for the future. *Edited by* K S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp 163-169.

Vaughn, C.C., Taylor, C.M., and Eberhard, K.J. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. *In* Conservation and management of freshwater mussels II. Initiatives for the future. *Edited by* K.S. Cummings, A.C. Buchanan, C.A. Mayer, and T.J. Naimo. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp. 157-162. Available from Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Illinois 61820 USA.

Wentworth, C.K. 1922. A scale of grade and class terms for clastic sediments. Journal of Geology, 30: 377-392.

Wilcox, D.B., Anderson, D.D., and Miller, A.C. 1993. Survey protocol and decision criteria for estimating the likelihood that *Lampsilis higginsi* is present in areas within the Upper Mississippi River System. *In* Conservation and management of freshwater mussels. Proceedings of the Upper Mississippi River Conservation Committee Symposium, St. Louis, Missouri. *Edited by* K.S. Cummings, A.C. Buchanan, and L.M. Koch. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp. 163-167.

9.0 ADDITIONAL READING

Ahlstedt, S. 1979. Recent mollusk transplants into the North York Holston River in southwestern Virginia. Bulletin of the American Malacological Union, Inc. pp.21-23.

Allan, J.D. 1995. Stream ecology, structure and function of running waters. Chapman & Hall, New York, NY.

Amyot, J.P., and Downing, J.A. 1991. Endo- and epibenthic distribution of the unionid mollusc *Elliptio complanata*. Journal of the North American Benthological Society 10:280-285.

Anthony, J.L., and Downing, J.A. 2001. Exploitation trajectory of a declining fauna: a century of freshwater mussel fisheries in North America. Can. J. Fish. Aquat. Sci. 58: 2071–2090.

Baker, F.C. 1928. Freshwater Molluscs of Wisconsin. Part II. Pelecypoda. Bulletin of the Wisconsin Geological and Natural History Survey. Vol. 70. 495 p.

Bogan, A.E. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. Am. Zool. 33:599-609.

Bolden, S.R., and Brown, K.M. 2002. Role of stream, habitat, and density in predicting translocation success in the threatened Louisiana pearlshell, *Margaritifera hembeli* (Conrad). J. N. Am. Benthol. Soc., 2002, 21(1):89–96

Bowen, Z.H., Malvestuto, S.P., Davies, W.D., and Crance, J.H. 1994. Evaluation of the mussel fishery in Wheeler Reservoir, Tennessee River. J. Freshwat. Ecol. 9:313-319.

Buchanan, A.C. 1980. Mussels (naiades) of the Meramec River basin. Missouri. Aquatic Series No. 17, Missouri Department of Conservation, Jefferson City, MO. 68 p.

Carlander, H. B. 1954. Mussel fishing and the pearl button industry. *In* A history of fish and fishing in the Upper Mississippi River. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp. 40-51.

Claassen, C. 1994. Washboards, pigtoes, and muckets: historic musseling in the Mississippi watershed. Hist. Archaeol. 28:1-145.

Clark, C.F. 1977. The freshwater naiads of Ohio, Part I. St. Joseph River of the Maumee. Sterkiana 65/66: 14-36.

Clarke, A.H. 1981. The freshwater Mollusca of Canada. Canadian Museum of Nature, Ottawa, Ontario, Canada.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2003*a*. COSEWIC assessment and update status report on the kidneyshell *Ptychobranchus fasciolaris* in Canada. Committee on the Status of Endangered Wildlife in Canada. vi + 32 pp.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2003*b*. COSEWIC Assessment and Status Report on the Round Hickorynut *Obovaria subrotunda* in Canada. Committee on the Status of Endangered Wildlife in Canada. vi + 32 pp.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2004. COSEWIC assessment and status report on the Round Pigtoe *Pleurobema sintoxia* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 33 pp.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). 2005. COSEWIC Species Database. Available: http://www.COSEWIC.gc.ca. Accessed: July 3, 2005.

Cope, W.G., and Waller, D.L. 1995. Evaluation of freshwater mussel relocation as a conservation and management strategy. Regulated Rivers: Research and Management 11:147-155.

Cope, W.G., Hove, M.C., Waller, D.L., Hornbach, D.J., Bartsch, M.R., Cunningham, L.A., Dunn, H.L., and Kapuscinski, A.R. 2003. Evaluation of relocation of unionid mussels to insitu refugia. Journal of Molluscan Studies 69: 27-34.

Cummings, K.S., and Mayer, C.A. 1992. Field Guide to Freshwater Mussels of the Midwest. Illinois Natural History Survey Manual 5. 194 p.

Dennis, S.D. 1984. Distributional analysis of the freshwater mussel fauna of the Tennessee River system, with special reference to possible limiting effects of siltation. Ph.D. thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 245 p.

Dextrase, A., Metcalfe-Smith, J., DiMaio, J., Sutherland, D., Zammit, A., Holm, E., and Ciuk, M. 2001. Species at risk in the Sydenham River. Background report for the Sydenham River Recovery Team. 45 p. Available from: http://www.sydenhamriver.on.ca/Publications/Fishes%20at%20Risk%20in%20the%20Sydenham%20River%20Watershed.pdf

Downing, J.A., Amyot, J.P., Perusse, M., and Rochon, Y. 1989. Visceral sex, hermaphroditism, and protandry in a population of the freshwater bivalve *Elliptio complanata*. Journal of the North American Benthological Society 8:92-99.

Dunn, H.L., Sietman, B.E., and Kelner, D.E. 2000. Evaluation of recent unionid (Bivalvia) relocations and suggestions for future relocations and reintroductions. Proceedings of the first Freshwater Mollusk Conservation Society symposium. Ohio Biological Survey, Columbus. pp. 169-183.

Elder, J.F., and Collins, J.J. 1991. Fresh-water mollusks as indicators of bioavailability and toxicity of metals in surface-water systems. Reviews of Environmental Contamination and Toxicology 122:37-79.

Gendron, A.D. 1999. Status report on the Mudpuppy, *Necturus maculosus*, in Canada. Prepared for the Committee On the Status of Endangered Wildlife in Canada. 86 p.

Gordon, M. E., and Layzer, J.B. 1989. Mussels (Bivalvia: Unionidae) of the Cumberland River: Review of life histories and ecological relationships. U. S. Fish and Wildlife Service, Biological Report 89(15): 1-99. (cited in Layzer and Gordon 1993).

Green, R.H., Hinch, S.G., Metcalfe, J.L., and Young, V.H. 1989. Use of fresh- water mussels (Bivalvia, Unionidae) to monitor the nearshore environment of lakes. J. Great Lake. Res. 15:635-644.

Hamilton, H., Box, J. B., and Dorazio, R.M. 1997. Effects of habitat suitability on the survival of relocated freshwater mussels. Regulated Rivers Research Management 13: 537-541.

Hanson, J.M., Mackay, W.C., and Prepas, E.E. 1988*a*. The effects of water depth and density on the growth of a unionid clam. Freshwat. Biol. 19:345-355.

Kat, P.W. 1982. Effects of Kat, P.W. 1982. Effects of population density and substratum type on growth and migration of *Elliptio complanata* (Bivalvia: Unionidae). Malacological Rev., 15, 19–127

Layzer, J.B., and Gordon, M.E. 1993. Reintroduction of mussels into the Upper Duck River, Tennessee. *In* Conservation and management of freshwater mussels. Proceedings of the Upper Mississippi River Conservation Committee Symposium, St. Louis, Missouri. *Edited by* K.S. Cummings, A.C. Buchanan, and L.M. Koch. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp. 89-92.

Mackie, G.L. 2000*a*. Mollusc introductions through aquarium trade. *In* Non-indigenous freshwater organisms: vectors, biology and impacts. *Edited by* R. Claudi, and J. Leach. Lewis Publishers, Boca Raton, Florida. pp. 135-150.

Mackie, G.L. 2000*b*. Ballast water introductions of Mollusca. *In* Non-indigenous freshwater organisms: vectors, biology and impacts. *Edited by* R. Claudi, and J. Leach. Lewis Publishers, Boca Raton, Florida. pp. 255-272.

Mackie, G.L. 2000*c*. Introduction of molluscs through the import for live food. *In* Non-indigenous freshwater organisms: vectors, biology and impacts. *Edited by* R. Claudi, and J. Leach. Lewis Publishers, Boca Raton, FL. pp. 305-314.

Mackie, G.L. 2002. Traits of endangered and invading freshwater molluscs in North America. *In* Alien invaders in Canada's waters, wetlands, and forests. *Edited by* Claudi, R.,P. Nantel, E. Muckle-Jeffs. Publ., Can. Forest Serv., Nat. Resources Canada, Ottawa, Ontario. pp. 187-198.

Master, L.L., Stein, B.A., Kutner, L.S., and Hammerson, G.A. 2000. Vanishing assets: conservation status of U.S. species. *In* Precious heritage: the status of biodiversity in the United States. Edited by B. A. Stein, L. S. Kutner, and J. S. Adams. Oxford University Press, New York. pp. 93-118.

McNichols, K, Mackie, G.L., and Ackerman J. 2004. Fish host determination of endangered freshwater mussels in the Sydenham River Ontario, Canada. ESRF 2004/2005 Final Report. 25 p.

Metcalfe, J.L., and Charlton, M.N. 1990. Fresh-water mussels as biomonitors for organic industrial contaminants and pesticides in the St. Lawrence River. Sci. of the Total Environ. 97/98:595-615.

Metcalfe-Smith, J.L., and Cudmore-Vokey, B. 2004. National general status assessment of freshwater mussels (Unioniacea). Environment Canada. National Water Research Institute. Burlington, Ontario, Canada. NWRI Contribution No. 04-027. 37 pp. + Appendices.

Metcalfe-Smith, J.L., Staton, S.K., Mackie, G.L., and West, E.L. 1998. Assessment of the current conservation status of rare species of freshwater mussels in southern Ontario. Environment Canada, National Water Research Institute. Burlington, Ontario. NWRI Contribution No. 98-019. 85 p.

Metcalfe-Smith, J.L., Staton, S.K., Mackie, G.L., and Scott, I.M. 1999. Range population and environmental requirements of rare species of freshwater mussels in southern Ontario. Environment Canada. National Water Research Institute. Burlington, Ontario. NWRI Contribution No. 99-058. 92 p.

Metcalfe-Smith, J.L., Mackie, G.L., Di Maio, J., and Staton, S.K. 2000. Changes over time in the diversity and distribution of freshwater mussels (Unionidae) in the Grand River, southwestern Ontario. J. Great Lake. Res. 26(4): 445-459.

Metcalfe-Smith, J.L., Di Maio, J., Staton, S.K., and de Solla, S.R. 2003. Status of the freshwater mussel communities of the Sydenham River, Ontario, Canada. American Midland Naturalist 150: 37-50.

Metcalfe-Smith, J.L., McGoldrick, D.J., Williams, M., Schloesser, D.W., Biberhofer, J., Mackie, G.L., Arts, M.T., Zanatta, D.T., Johnson, K., Marangelo, P., and Spencer, T.D. 2004. Status of a refuge for native freshwater mussels (Unionidae) from the impacts of the exotic zebra mussel (*Dreissena polymorpha*) in the delta area of Lake St. Clair. Environment Canada, National Water Research Institute, Burlington, Ontario. Technical Note No. AEI-TN-04-001. 50 p.

Metcalfe-Smith, J.L., MacKenzie, A., Carmichael, I., and McGoldrick, D. 2005. Photo field guide to the freshwater mussels of Ontario. St. Thomas Field Naturalist Club Incorporated, St. Thomas, ON, Canada ISBN 0-9733179-2-2.

Morgan, A., Welker, N.J., and Lazer, J.B. 1993. Feasibility of reintroducing threatened and endangered mussels into Shoal Creek in Alabama and Tennessee. *In* Conservation and management of freshwater mussels II. Initiatives for the future. *Edited by* K.S. Cummings, A.C. Buchanan, C.A. Mayer, and T.J. Naimo. Upper Mississippi River Conservation Committee, Rock Island, Illinois. pp. 196-204.

Morris, T.J. 1996. The unionid fauna of the Thames River drainage, southwestern Ontario. Prepared for Aquatic Ecosystems Branch, Ontario Ministry of Natural Resources. 59 p.

Morris, T.J. 2006. Recovery strategy for the Wavyrayed Lampmussel (*Lampsilis fasciola*, Rafinesque 1820). *In* Canada. In Species at Risk Act Recovery Strategy Series. Ottawa. Fisheries and Oceans Canada. 51 p.

Morris, T.J., and Di Maio, J. 1998. Current distributions of mussels in rivers of southwestern Ontario. Malacological Review. 31(1): 9-17.

Morris, T.J., McGoldrick, D.J., and Metcalfe-Smith, J.L. 2005. Assessing characteristics of recovery: A case study using the endangered Wavyrayed Lampmussel (*Lampsilis fasciola*, Rafinesque 1820). Canadian Science Advisory Secretariat Research Document 2005/062.

Naimo, T.J. 1995. A review of the effects of heavy metals on fresh-water mussels. Ecotoxicology 4:341-362.

Nalepa, T.F., Gardner, W.S., and Malczyk, J.M. 1991. Phosphorus cycling by mussels (Bivalvia) in Lake St. Clair. Hydrobiologia 219:239-250.

Newton, T.J., Monroe, E.M., Kenyon, R., Gutreuter, S., Welke, K.I., and Thiel, P.A. 2001. Evaluation of relocation of unionids mussels into artificial ponds. Journal North American Benthological Society 20: 468-485.

Oesch, R.D. 1984. Missouri Naiades. A Guide to the Mussels of Missouri. Missouri Department of Conservation, Jefferson City, Missouri. 270 p.

Ortmann, A.E. 1919. A monograph of the naiades of Pennsylvania. Part III: Systematic account of the genera and species. Memoirs of the Carnegie Museum V.8. 384 p.

Richardson, T.D., and Yokely, P. 1996. A note on sampling technique and evidence of recruitment in freshwater mussels (Unionidae). Arch. Hydrobiol. 137:135-140.

Rogers, S.O., Watson, B.T., and Neves, R.J. 2001. Life history and population biology of the endangered tan riffleshell (*Epioblasma florentina walkeri*) (Bivalvia: Unionidae). Journal of the North American Benthological Society 20:582-594.

Sheehan, R.J., Neves, R.J., and Kitchel, H.E. 1989. Fate of freshwater mussels transplanted to formerly polluted reaches of the Clinch and North Fork Holston Rivers. J. Freshwat. Ecol. 5: 139-149.

Sherman, R.A. 1994. Life history information critical to the management of the state endangered Snuffbox Mussel, *Epioblasma triquetra* (Bivalvia: Unionidae) in Michigan. M.Sc. thesis, School of Natural Resources and the Environment, University of Michigan, Ann Arbor, Michigan. 40 p.

Smith, D.R., Villella, R.F., Lemarie, D.P., and von Oettingen, S. 2000. How much excavation is needed to monitor freshwater mussels? Proceedings of the first Freshwater Mollusk Conservation Society symposium. *Edited by* P.D. Johnson and R.S. Buder. Ohio Biological Survey, Columbus. pp. 203-218.

Smith, D.R., Villella, R.F., and Lemarie, D.P. 2003. Application of adaptive cluster sampling to low-density populations of freshwater mussels. Environ. Ecol. Stat. 10:7-15.

Stanfield, L., and Kuyvenhoven, R. 2005. Protocol for applications used in the Aquatic Landscape Inventory Software application for delineating, characterizing and classifying valley segments within the Great Lakes basin. Ontario Ministry of Natural Resources Report, July 27, 2005.

Staton, S.K., Dextrase, A., Metcalfe-Smith, J.L., Di Maio, J., Nelson, M., Parish, J., Kilgour, B., and Holm, E. 2003. Status and trends of Ontario's Sydenham River ecosystem in relation to aquatic species at risk. Environ. Monit. Assess. 88:283-310.

Strayer, D. 1983. The effects of surface geology and stream size on freshwater mussel distribution in southeastern Michigan, USA. Freshwat. Biol. 13:253-264.

Strayer, D.L., and Smith, L.C. 1996. Relationships between zebra mussels (*Dreissena polymorpha*) and unionid clams during the early stages of the zebra mussel invasion of the Hudson River. Freshwat. Biol. 36:771-779.

Strayer, D.L., and Fetterman, A.R. 1999. Changes in the distribution of freshwater mussels (Unionidae) in the upper Susquehanna River basin, 1955-1997. American Midland Naturalist 142:328-339.

Strayer, D.L., Cole, J.J., Likens, G.E., and Buso, D.C. 1981. Biomass and annual production of the freshwater mussel Elliptio complanata in an oligotrophic softwater lake. Freshwat. Biol. 11:435-440.

Strayer, D.L., Hunter, D.C., Smith, L.C., and Borg, C. 1994. Distribution, abundance, and role of freshwater clams (Bivalvia: Unionidae) in the freshwater tidal Hudson River. Freshwat. Biol. 31:239-248.

Strayer, D.L., Caraco, N.F., Cole, J.J., Findlay, S., and Pace, M.L. 1999. Transformation of freshwater ecosystems by bivalves: a case study of zebra mussels in the Hudson River. BioScience 49:19-27.

Trdan, R.J., and Hoeh, W.R. 1993. Relocation of two state-listed mussel species (*Epioblasma torulosa rangiana* and *Epioblasma triquetra*) in Michigan. *In* Conservation and management of freshwater mussels. Proceedings of the Upper Mississippi River Conservation Committee Symposium, St. Louis, Missouri. *Edited by* K.S. Cummings, A.C. Buchanan, and L.M. Koch. Illinois Natural History Survey, Champaign, Illinois. pp. 100-105

van der Schalie, H. 1938. The naiad fauna of the Huron River, in southeastern Michigan. Miscellaneous Publications of the Museum of Zoology, University of Michigan 40:1-83.

Vaughn, C.C., and Hakenkamp, C.C. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwat. Biol. 46:1431-1446.

Villellalla, R.F., King, T.L., and Starliper, C.E. 1998. Ecological and evolutionary concerns in freshwater bivalve relocation programs. Journal of Shellfish Research 17: 1407-1413.

Waller, D.L., Rach, J.J., and Cope, W.G. 1995. Effects of handling and aerial exposure on the survival of freshwater mussels. J. Freshwat. Ecol. 10: 199-207.

Waller, D.L., Gutreuter, S., and Rach, J.L. 1999. Behavioral responses to disturbance in freshwater mussels with implications for conservation and management. Journal of the North American Benthological Society: 18 (3): 381–390.

Watson, E.T., Metcalfe-Smith, J.L., and DiMaio, J. 2001. COSEWIC status report on the snuffbox Epioblasma triquetra. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 33 p.

Welker, M., and Walz, N. 1998. Can mussels control the plankton in rivers? A planktological approach applying a Lagrangian sampling strategy. Limnol. Oceanogr. 43:753-762.

West, E.L., Metcalfe-Smith, J.L., and Staton, S.K. 2000. Status of the rayed bean, *Villosa fabalis* (Bivalvia: Unionidae), in Ontario and Canada. Can. Field. Nat. 114(2): 248-258.

Wetzel, R.G., and Likens, G.E. 1995. Limnological analyses. Springer-Verlag, New York, NY.

Williams, J.D., Warren, Jr., M.L., Cummings, K.S., Harris, J.L., and Neves, R.J. 1993. Conservation status of the freshwater mussels of the United States and Canada. Fisheries. 8(9): 6-22.

Woolnough, D.A. 2002. Life history of endangered freshwater mussels of the Sydenham River, southwestern Ontario, Canada. Unpublished Master's thesis, University of Guelph, Guelph, Ontario.

Zanatta, D.T., Mackie, G.L., Metcalfe-Smith, J.L., and Woolnough, D.A. 2002. A refuge for native freshwater mussels (Bivalvia: Unionidae) from impacts of the exotic zebra mussel (*Dreissena polymorpha*) in Lake St. Clair. J. Great Lake. Res. 28(3): 479-489.

Ziuganov, V., Zotin, A., Nezlin, L., and Tretiakov, V. 1994. The freshwater pearl mussels and their relationships with salmonid fish. VNIRO Publishing House, Moscow.

APPENDIX 1: GUIDELINES FOR FILLING OUT AN APPLICATION FOR A *SARA* PERMIT WITH AN EXAMPLE OF A COMPLETED FORM

OBTAINING A *SARA* PERMIT

Although it is illegal to kill, harm, harass, capture, or take any endangered or threatened species protected under the *Species at Risk Act* (*SARA*), the Minister of Fisheries and Oceans may permit activities which could affect an aquatic species protected under the *Act* if he believes these activities will not jeopardize the survival or recovery of species at risk. It is imperative to show that: the activity is a survey relating to the conservation of the species and is conducted by qualified persons; the activity benefits the species or is required to enhance its chance of survival in the wild; or affecting the species is incidental to carrying out the activity. If you are planning to carry out activities that may impact a species that is protected under *SARA*, you will require a *SARA* permit. As a proponent of a scientific survey project, you will need to:

- be proactive in your survey design,
- demonstrate awareness of the provisions of *SARA*, and
- demonstrate that measures are being taken to minimize harm to listed species and that the best solution has been adopted.

For more information on the the *SARA* permit, visit:
http://www.dfo-mpo.gc.ca/species-especes/permits/sarapermits_e.asp

To download the permit in PDF or Word format, go to:
http://www.dfo-mpo.gc.ca/species-especes/permits/saraapplication_e.asp

GUIDELINES FOR FILLING OUT THE APPLICATION

Since the permit encompasses activities related to mussel SAR, it is recommended that the lead investigator of these activities apply for the permit so that DFO is informed of the expertise of the people handling the mussel SAR. *DFO will require two to three weeks to process the permit so submit the application at least a month before the activities are to begin.*

Section 1, Applicant Information:

Basic information about the applicant. Provide a brief description of relevant experience and credentials (education, degrees, and diplomas). Be sure to indicate your expertise with freshwater mussels and that the experts will be on site for all activities. Provide contact information where you can be reached in the days and weeks following your application in case clarifications are required.

Section 2, Logistics of Proposed Research:

2A Lead Investigator: Provide the name of the person who oversees the mussel SAR activities.

2B Other researchers: Include the names of other lead researchers, for example, the field leader and other people with mussel research experience.

2C Vessel/Platform: Not applicable unless operating from a boat, barge, etc.

2D Locations and dates where research will be done: Provide dates to cover entire period, including dates for relocating and monitoring studies.

2E SARA Species to be included in permit: It is important to list all *SARA*-listed species that may occur in the proposed research area and the anticipated mortalities for each. Be as specific as possible but for any relocation work the intent is to keep mortality to a minimum. For information on the distribution and known localities for listed species in your area, contact the Regional DFO person.

Section 3, Description of Proposed Research and Potential Impacts on *SARA* listed species:(Note: A detailed project work plan or proposal should be attached to this section).

3A Objective/Purpose of Research

For presence/absence surveys for mussels, the purpose is usually to identify the freshwater mussel species and their relative abundances within a prescribed search area (PSA). If relocation of mussels is planned, the objective is usually to collect and relocate mussels from a PSA, where habitat and species will be impacted by an activity (e.g., bridge, pipeline, culvert, construction, etc.), to an upstream location that has similar species composition, habitats, and fish hosts. If monitoring of survival and growth is planned, the objective is to ensure that impacts of the relocation are negligible to minimal on survival and growth of both the transplanted mussels and on the mussel community existing at the relocation site.

3B Briefly explain field/study techniques

Make sure the activities described here relate to the purpose/objectives stated above. Give the size of the PSA. If the study is to detect the presence of SAR, provide an estimate of the proportion of the PSA to be searched for mussels and habitat types. State how you plan to search the proportion (e.g., random or stratified random searches). Briefly describe the methodology (e.g., visual/tactile search, quadrat search), the amount of effort (e.g., number of people, person hours, number of quadrats), and any special techniques to increase chances of

finding SAR (e.g., use of polarized sunglasses, 'raccooning', viewing boxes, depths of sediments to be searched, sieves to find juveniles). State what you plan to do with the collected mussels (e.g., return to natural habitat immediately, temporarily maintain in containers, type of container).

If it is a relocation activity, the entire PSA should be searched for mussels. State what criteria were used in selecting the relocation site. Briefly describe how you will ensure that the entire PSA will be searched (e.g., marking PSA off into 1 m wide rows and then flipping 1 m^2 quadrats within rows, 1 m at a time from the upstream end of the PSA to the downstream end). Briefly describe the amount of effort (e.g., number of people, person hours, number of quadrats), and any special techniques to increase chances of finding SAR (e.g., use of polarized sunglasses in clear water, 'raccooning' in turbid water, viewing boxes, depths of sediments to be searched, sieves to find juveniles).

State if you plan to measure survival and growth of mussels and if so, how (e.g., providing each mussel with a coded shellfish tag, measuring length, width, height). Describe how you plan to maintain mussels while they are being coded and measured (e.g., in containers with holes to allow constant supply of river water). Leave details of coding, container types and sizes, survival and growth methodology, etc. for the work plan.

3C Describe anticipated or potential disturbances to each of the SARA-listed species, impacts on habitats used by the species. In this section, list the species, the nature of harm to each, and the likelihood of harm or encounters (high, medium, low). State why the mussels will be disturbed (e.g., they have to be removed from their habitat in order to identify and enumerate them); how they will be disturbed (e.g., removed from substratum temporarily to identify and enumerate species); how you plan to minimize the impact on the species (e.g., use fingers to remove, not grabs or rakes, which may harm shells); how long the mussel will be out of water and exposed to the atmosphere (e.g., 3 to 5 minutes maximum); how you plan to replace them into the substrate (e.g., same burrow, different burrow but similar substrate, posterior end up). Describe any potential impacts on the habitats used by the species; if a relocation study, briefly describe the impacts of construction activities on the habitats and the reason(s) for relocation (e.g., coffer dams will be built and PSA will be dewatered which will kill any mussels in the PSA). Describe any potential impacts of transplanted mussels on native mussels in the relocation site and how you plan to minimize those impacts (e.g., by selecting a site with same species composition and fish hosts, an area larger than the PSA to minimize crowding, and monitoring survival and growth of relocated mussels and native mussels in the relocation site).

Section 4, Following from the criteria in Section 73 of *SARA*; if impacts on a listed species are likely, the proponent should specify:

This section addresses information specific to Section 73 (3) pre-conditions in *SARA*. Pre-conditions (a) and (b) require that alternatives and measures such as area and/or time changes or changes to sampling gear or techniques must be considered and implemented if feasible before a permit or authorization can be issued. For pre-condition (c), indicate to the best of your knowledge whether the proposed research activity, given 4A and 4B, will jeopardize survival or recovery of the species.

4A What alternatives to the proposed method of conducting the activity have you considered? How is the chosen method the best solution to reduce impact to the species?

In addressing these questions consider the impacts of classical methods for collecting benthic organisms (e.g., Ponar grabs, Peterson grabs, Surber samplers) and their impact or harm to the species, their efficacy of collecting large mussels, and their efficacy of estimating mussel density and diversity. Give the advantages of hand picking vs. mechanical 'picking' with respect to potential harm or other impacts on the species.

4B What mitigation measures have been included, and how do they minimize the potential impacts on listed species and/or habitats? What mitigation measures have been considered and not included, and for what reasons were they rejected?

In addressing these questions, consider the goals and objectives of relocation of mussels, for example, to remove them from a high probability of harm to one of low to no likelihood of harm. The entire procedure, from collecting to relocating mussels, stresses the use of methods designed to cause the least harm to the animals and to provide best estimates of density and diversity in a variety of size classes. Stress the advantages of collecting mussels by hand versus grab samplers and suction devises, the probability of damage to shells by mechanical devices, and the efficacy of mechanical devises at estimating density and diversity of small and large mussels. Consider the utility of mechanical devices at sampling mussels under boulders, in sand and gravel trapped between rocks, or in thick *Potamogeton* and *Cladophora* beds that occur in most rivers in southern Ontario.

APPENDIX 1 (cont'd): EXAMPLE OF A COMPLETED APPLICATION FOR A *SARA* PERMIT

Fisheries and Oceans Canada | Pêches et Océans Canada

Application for a Species at Risk Permit
Scientific Research/Education
Fisheries & Oceans Canada

1 Applicant Information:

Name:	Applicant 1, Applicant 2		
Organization:	XXX Company		
Address:			
Street:	XXX St.		
City:	Town		
Province/State:	ON		
Country:	Canada	Postal Code/Zip:	X#X #X#
Phone:	(905)###-####	Cellular:	Fax:
Email:			

Applicant Experience/Credentials: Applicant 1- X years working with molluscs, Ph.D. since 19XX, professor XXX University 19XX-19XX; Applicant 2- B.Sc. currently working on MSC, XXX University, specialist in unionid identification X yrs.

2. Logistics of Proposed Research:

A Lead Investigator	Applicant 1
B Other researchers	List names of other researchers here.
C Vessel / Platform Name:	
CFV/Registration #:	
Country of Registration:	

D Locations and dates where research will be done	XXX River, immediately south of bridge at Highway ##; mussels to be collected and relocated MM/DD/YY to MM/DD/YY, or as soon as *SARA* permit is obtained.

E *SARA* Species to be included in Permit	**Anticipated # of mortalities for each species**
Lampsilis fasciola, Epioblasma triquetra, Pychobranchus fasciolaris, Obovaria subrotunda, Pluerobema sintoxia	Zero for all mussel species

3. Description of Proposed Research and Potential Impacts on *SARA* listed species:

A Objective/Purpose of Research

The goal of the activities is to relocate mussel species at risk in the XXX River where a site investigation for a new bridge will be conducted adjacent to an existing bridge at Highway ## (i.e. North ##o ##.###' West ##o ##.###) in XXtown, Ontario. The site investigation involves drilling four boreholes into the river bed. No bridge construction will occur at this stage; the actual bridge construction may not occur for 1 to 2 years time. This is a Ministry of XXX Project and X Consulting is the subconsultant for the project. The Prime Consultant is XXX Limited. They also have an environmental subconsultant on the team and X Consulting will do the drilling and co-ordinate the mussel surveys.

B Briefly explain field collections/study techniques

The exact location of the boreholes will be in line with the 2 existing piers that are founded in the river. One of the piers is in the middle of the river and the other is near the west bank. The boreholes will be about 8" in diameter through the riverbed, and will be drilled to a depth of approximately 12 m (40 feet) below the riverbed surface. The drill rig will be situated on a barge, the dimensions of which will be approximately 7.3 m long by 5.5 m wide. The barge will either be floating and anchored to the shore with cables, or 2 to 4 spuds of approximately 15-30 cm (6" to 12") diameter will be used to anchor into the riverbed. Therefore, at each borehole location, they are utilizing about 40 square meters of space (the area of the barge) and a maximum of 5 small diameter holes will penetrate the river bed.

Including the area to be traversed by the barge across the river to each bore hole, the anticipated area to be impacted directly by drilling activities is approximately 5.5 m (width of barge) x 60 m (width of river) = 330 m^2. Two crossings need to be made so the area to be directly impacted by the barge is approximately 660 m^2. Assuming there will be minimal impact adjacent to the barge, the zone of influence will be minimal. Average summer water depth at the site is about 0.4 m; maximum summer water depth is apparently 1.5 m, but if deeper we will use three certified SCUBA divers to sample deep sites. All searches for mussels will be conducted by an 8-person team using waders, polarized sunglasses (which greatly help to visually locate any mussels in clearer waters), square meter quadrats, sieves with 7 mm mesh openings, and/or clam scoops with 7 mm mesh openings and a 2 m long handle. Deeper areas and more turbid areas will be searched using Lucite-bottom viewing boxes and/or long-handled clam scoops with 0.7 mm mesh openings.

Eight people will search for mussels until about 100 mussels have been collected. The mussels will be temporarily maintained in 20 L buckets with holes drilled into the sides so fresh river water can constantly flow through and bathe the mussels. Then three people will begin gathering information on the mussels: one to attach a coded Hallprint Shellfish tag to the shell; one to measure width (side to side), length (anterior to posterior) and height (top to bottom) of each shell; one to record. The only time a mussel will be out of water is when it is being measured and coded (~2-3 minutes). The remaining five people will continue collecting mussels and delivering them to the three people for marking and measuring.

The search will be conducted for both adult and juvenile mussels. Sediments will be collected with scoops or small shovels and placed into sieves with 7 mm mesh openings to search for juveniles. All juveniles and mussels less than 15 mm will be temporarily stored in pails with 4 mm holes drilled in the sides; the pails will be placed in the river so that river water can constantly bathe the mussels. Any mussels larger than 15 mm found on the sieves or collected by hand will be temporarily maintained in 20 L buckets with 13 mm holes drilled in the sides so fresh river water will constantly bathe the mussels. 'Fruit pickers' (long tongs with rubber pads so as not to damage shells) will be used to remove adult mussels at the 1 m deep sites or sieves (with 7 mm mesh openings) on long handles will be used to scoop sediments to search for juveniles. Viewing boxes that are 40 and 60 cm deep (16" and 24") will also aid in locating mussels in more turbid and/or deeper sites.

All specimens of species at risk (SAR) will be coded and measured so we can monitor their growth and survival. All mussels, including common species, will be relocated, but only about 20-25% of the shells of common species will be marked and measured so we can monitor their growth and survival in the transplanted habitat. We will also code and measure about 20-25% of the mussels from the relocation site so we can monitor their growth and survival.

C Describe anticipated or potential disturbances to each of the *SARA*-listed species in **2E,** include impacts on habitat(s) used by the species: List the species, the nature of harm, and the likelihood of harm or encounters (High, Medium, Low)

The mussels will be disturbed during surveys because they must be removed from their habitat in order to identify, code, measure, and relocate them. We will begin at the downstream end of the prescribed search area and work upstream. The likelihood of harm is low for all species since our goal is to relocate mussels removed from the prescribed search area.

Collecting will begin downstream, before any drilling activity has begun. The river will be divided into two sections (A, B), each section having 60 rows, each row being 1 m wide by 5.5 m long. The rows will be used to guide the placement of the quadrats. It is recommended that all mussels collected and measured from the first section (A) be transplanted before collecting from the upstream section (B) so as not to stress the mussels already picked from the first section. The less time the mussels are kept in pails of water, the better. This will ensure that the entire area is searched in a systematic manner with the 1 m^2 quadrat. The mussels will be transplanted in a stratified random fashion, with all coded mussels randomly placed in one area and all uncoded mussels randomly placed in another area. This will aid in finding the marked mussels for the monitoring study. To further aid in relocating mussels, we will use brightly coloured Hallprint Shellfish tags glued to the posterior part of the shell. In addition, coloured tent pegs will be driven into the river bed beside each coded mussel to help locate coded 'native' and 'transplanted' mussels. Mussels will be buried in their normal positions, that is, with posterior end sticking out of the sediments

NOTE: Please attach the project workplan/proposal to this application

4. Following from the criteria in Section 73 of *SARA*; if impacts on a listed species are likely, the proponent should specify:

A What alternatives to the proposed method of conducting the activity have you considered? How is the chosen method the best solution to reduce impact to the species?

The classical methods (Surber sampler, Ponar grab, Peterson grab, etc.) for collecting benthic organisms either take too small a sample to reliably sample mussels or they are likely to cause harm to the mussels. Hand picking is by far the best method to keep the likelihood of harm low.

B What mitigation measures have been included, and how do they minimize the potential impacts on listed species and/or habitats? What mitigation measures have been considered and not included, and for what reasons were they rejected?

Unionids are best collected by hand, not by grabs or suction devices. Grabs have powerful jaws which could crush thin-shelled species and juveniles. Suction devices would collect juveniles and light mussels but not heavier, thick-walled species like *Actinonaias*. Neither devices are good for accurately determining presence because the areas sampled are less than 250 cm^2. Much of the bottom of the XXX River at the bridge site is sand, gravel, boulders, and cobble. Also, there are small to extensive beds of aquatic plants, not easily sampled by standard sampling devices. The plant beds will have to be removed by hand in order to examine the sediments below.

C	Will the sampling program jeopardize survival or recovery of the species, in light of responses to 4A and 4B above? If not, why not?
	No. Our goal is to determine if mussel species at risk are present in the prescribed search area. If species at risk are present, we expect to relocate the mussels from the prescribed search area to a site immediately upstream with similar habitat features. The relocation activities are to be done immediately before drilling begins. All activities are aimed at reducing impact on mussels and ensuring a low level of harm to the mussel community.

For internal use only

5.	**Jurisdictional Science evaluation of the application (complete ONE option):** ***to be completed by DFO Science***
	a) The proposed research complies with the conditions of Section 73 and may proceed: b) The proposed research would comply with the conditions of Section 73 and may proceed only if the following additional conditions are met (specify): c) The proposed research cannot proceed within the conditions of Section 73 for the following reasons: d) The information in the application is **inadequate** to assess the potential impacts of the proposed research on *SARA* species. Additional information is required on (specify): Signature of Jurisdictional Science Authority:

6. If Option a) is met, or the applicant agrees to the conditions in Option b), then the Jurisdictional Science Authority should initial each of the conditions below which apply to the proposed research:	
A	The activity is scientific research relating to the conservation of *SARA*-listed species and will be conducted by qualified persons
B	The activity benefits the *SARA*-listed species or is required to enhance its chance of survival in the wild
C	Affecting the *SARA*-listed species is incidental to the carrying out the activity (Note—Fisheries Management may choose to evaluate the project on this condition, particularly for research not directed at the *SARA* species listed in 3C)

For office use

Licence Type:	*Scientific Trans*	*DFO Processing Officer*	
Date Received		*Date Required*	
Sara Species		*Permit #*	
Cites Required		*Animal Care Permit Required*	
Previous Permit #		*Previous Report on File:*	

Return Application to: *Regions to fill in*

APPENDIX 2: AN EXAMPLE OF A COMPLETED DATA SHEET

Date: August 1, 2006
Construction Site WC1

Code	Species	Length mm	Width mm	Height, mm	Relocated to:	Notes
A&B001	Spike (Elliptio dilata)	82.07	38.15	24.58	RS1	
A&B002	Spike	107.48	51.39	32.97	RS1	
A&B003	Wavyrayed Lampmussel (*Lampsilis fasciola*)	12.31	9.29	4.83	RS1	Photos taken
A&B003	Elktoe (*Alasmidonta marginata*)	66.44	39.78	25.69	RS1	
A&B004	Wabash Pigtoe (*Fusconaia flava*)	100.34	73.96	40.32	RS1	
A&B005	Wabash Pigtoe	84.25	61.71	30.97	RS1	
A&B006	Wabash Pigtoe	12.31	9.29	4.83	RS1	
A&B007	Wabash Pigtoe	22.15	16.20	8.23	RS1	
A&B008	Wavyrayed Lampmussel	42.10	28.10	17.07	RS1	Photos taken
Mussels from relocation site, RS1					**Put back into**:	
C&B001	Spike	100.48	49.39	30.97	RS1	
C&B002	Wavyrayed Lampmussel (*Lampsilis fasciola*)	11.31	9.00	4.55	RS1	Photos taken
C&B003	Elktoe (*Alasmidonta marginata*)	76.22	99.48	27.66	RS1	
C&B004	Wabash Pigtoe (*Fusconaia flava*)	90.11	53.98	33.32	RS1	
C&B005	Wabash Pigtoe	44.28	30.78	15.77	RS1	
Mussels from control site, CS1					**Put back into**	
D&E001	Elktoe (*Alasmidonta marginata*)	19.33	9.35	3.83	CS1	
D&E002	Wabash Pigtoe (*Fusconaia flava*)	93.84	46.74	28.86	CS1	
D&E003	Wabash Pigtoe	28.88	13.26	6.03	CS1	
D&E004	Wabash Pigtoe	22.50	10.78	4.57	CS1	
D&E005	Wabash Pigtoe	22.19	10.33	4.42	CS1	
D&E006	Wavyrayed Lampmussel	22.72	10.24	4.07	CS1	Photos taken

APPENDIX 2: AN EXAMPLE OF A COMPLETED DATA SHEET (cont'd)

Date: September 1, 2006
Construction Site WC1

Code	Species	Length mm	Width mm	Height, mm	Relocated to:	Notes
A&B001	Spike (Elliptio dilata)	82.07	38.15	24.58	RS1	
A&B002	Spike	107.48	51.39	32.97	RS1	
A&B003	Wavyrayed Lampmussel (*Lampsilis fasciola*)	13.55	11.20	5.33	RS1	Some growth
A&B003	Elktoe (*Alasmidonta marginata*)	66.44	39.78	25.69	RS1	
A&B004	Wabash Pigtoe (*Fusconaia flava*)	100.34	73.96	40.32	RS1	
A&B005	Wabash Pigtoe				RS1	Not found
A&B006	Wabash Pigtoe	15.36	10.39	5.83	RS1	Some growth
A&B007	Wabash Pigtoe	23.05	17.22	8.83	RS1	Some growth
A&B008	Wavyrayed Lampmussel	42.10	28.10	17.07	RS1	
Mussels from relocation site, RS1					**Put back into**	
C&B001	Spike	100.48	49.39	30.97	RS1	
C&B002	Wavyrayed Lampmussel (*Lampsilis fasciola*)	15.21	11.10	6.44	RS1	Some growth
C&B003	Elktoe (*Alasmidonta marginata*)	76.22	99.48	27.66	RS1	
C&B004	Wabash Pigtoe (*Fusconaia flava*)	90.11	53.98	33.32	RS1	
C&B005	Wabash Pigtoe	44.28	30.78	15.77	RS1	
Mussels from control site, CS1					**Put back into**	
D&E001	Elktoe (*Alasmidonta marginata*)	19.33	9.35	3.83	CS1	
D&E002	Wabash Pigtoe (*Fusconaia flava*)	93.84	46.74	28.86	CS1	
D&E003	Wabash Pigtoe					Not found
D&E004	Wabash Pigtoe	24.55	11.58	5.37	CS1	Some growth
D&E005	Wabash Pigtoe	23.99	11.63	5.52	CS1	Some growth
D&E006	Wavyrayed Lampmussel	23.42	10.94	4.57	CS1	Some growth